United States
Environmental Protection Agency

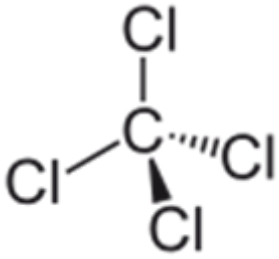
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Carbon Tetrachloride

Systematic Review Supplemental File:

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

CASRN: 56-23-5



October 2020

Table Listing

Acute (<24 hr)

Short-term (1-30 days)

Other

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 Roudabush et al., 1965 for an acute dermal toxicity and dermal irritation studies study on acute toxic-ity/poisoning and irritation outcomes**

Study Citation:	Roudabush, RL; Terhaar, CJ; Fassett, DW; Dziuba, SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs Toxicology and Applied Pharmacology, 7(4), 559-565
Data Type:	acute dermal toxicity and dermal irritation studies
HERO ID:	79743

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	The manufacturer was reported. The batch lot number for materials was not reported; however, this omission is unlikely to have a substantial impact on result.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity or chemical grade was not reported.; however given other information, purity was not expected to be of concern.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The use of controls were not discussed in the methodology sections of the report; however, the results table of the dermal irritation tests reported results for distilled water. The standard test guidelines (e.g., OECD) do not require negative controls for acute toxicity studies.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups for either the acute toxicity nor the irritation studies.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test materials were noted to be undiluted. Storage conditions were not reported; however, omission of these details are unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported for both studies and were administered consistently across study groups for both species.
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Study report does not specify the administered dermal doses for the acute toxicity study or the irritation study for either species. The report only states "a minimum of 3 dosages was employed" for the acute dermal toxicity test..
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The report notes that the procedure followed the protocols described in the Regulations (21 CFR 191.10), which includes details on exposure duration for both the acute dermal toxicity test (24 hours) and the primary irritation test (24 hours and 72 hours).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	The number of exposure groups was stated as "a minimum of 3 dosages" for the acute dermal toxicity study; the actual number of dose groups and spacing is not reported. The dosing of the irritation study is also not reported.

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Study Citation: Roudabush, RL; Terhaar, CJ; Fassett, DW; Dziuba, SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs Toxicology and Applied Pharmacology, 7(4), 559-565
Data Type: acute dermal toxicity and dermal irritation studies
HERO ID: 79743

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure was reported and appropriate for the study types
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	There are deficiencies in the reporting of the test animal characteristics. The strain of guinea pigs and rabbits, sex used for each study, and the starting body weight ranges were reported. There is some uncertainty in the source of white rabbits (reported to be from a "local supplier"). These uncertainties are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not 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 reported number of animals per study group for the acute toxicity test was unclear (reported to be "usually" 4 animals/dose group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of minor details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Details on outcome assessments sampling were not reported, but is likely that all tested animals were sampled.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The study types do not require blinding of assessors.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group (distilled water) was adequate for the dermal irritation study. There was no reported control used in the acute toxicity test.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were no reporting for any possible differences among the study groups that could influence the outcome assessment.; however, the lack of reporting is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data for outcomes unrelated to exposure for each study group were not reported, but the lack of reporting is unlikely to influence the study results.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Roudabush, RL; Terhaar, CJ; Fassett, DW; Dziuba, SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs Toxicology and Applied Pharmacology, 7(4), 559-565
Data Type:	acute dermal toxicity and dermal irritation studies
HERO ID:	79743

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not well described but would unlikely have a substantial impact on results. The acute toxicity test reported calculating the LD50 using the method of Finney (1952), while the calculation of the primary irritation score were made according to the Regulations (21 CFR 191.11).
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were reported for most, but not all, outcomes by exposure group. Data was reported for guinea pigs (male only) but was not reported by sex for rabbits, rather the data reported was for males and females combined. There was not presentation of mortality incidence for the acute toxicity study and no description of severity scores for the irritation study (only the primary irritation score was reported). These uncertainties in outcome reporting are unlikely to have substantial impact on results.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 2: **Animal toxicity evaluation results of Hayes et al., 1986 for an acute oral lethality study on mortality outcomes**

Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-l mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	acute oral lethality test in mice
HERO ID:	194400

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	Medium	× 1	2	Test substance source and lot number reported, but certification/analytical verification of identity was not.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance reported to be HPLC grade and >99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative control not required for acute lethality study.
	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 randomizing the mice but is not clear regarding the allocation.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Study reports daily preparation of solution, but does not report storage.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Only one exposure group tested
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose reported in mg/kg bw; body weight not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Single exposure group is not sufficient to determine LD50
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	10/sex were tested; this is more than adequate for acute lethality
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	Duration of post-exposure observation was not reported.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Only one group tested.
	Metric 18:	Sampling Adequacy	High	× 1	1	Mortality assessed in all exposed animals.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Mortality is not subjective
	Metric 20:	Negative Control Response	Not Rated	NA	NA	No negative control was used
Domain 6: Confounding / Variable Control						

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Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	acute oral lethality test in mice
HERO ID:	194400

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Only one group tested
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	acute lethality test; no other outcomes assessed
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical analysis not possible on single group
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Mortality data were not reported
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.1	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

[⋆] MWF = Metric Weighting Factor.

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

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

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

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

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

Table 3: **Animal toxicity evaluation results of Wahlberg and Boman 1979 for an acute percutaneous toxicity in guinea pig**

Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substances were identified; however, the test substances were lacking characterization details; unlikely to have a substantial impact on results.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substances were identified; did not include batch/lot numbers; unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	Low	× 1	3	purity or grade of test substances were not reported; possible impurities were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Distilled water was used as a concurrent control
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not rated/applicable; positive control was not indicated by 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	Low	× 1	3	There were no details of test substance preparation and/or storage conditions reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Single application to skin depot (31 cm2) and covered CK: Not 31 cm2. The solvents was administered to a skin depot area 3.1 cm2
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	applied concentrations were reported in ml; mean body weight was reported to estimate an administered dose.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	single application, covered, and observed for 35 d
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups for CCl4 = 2 and TCE=1; number of exposure groups and spacing were not justified by the author; Doses were considered adequate to address the purpose of the study for changes in body weight for both CCl4 and TCE; however for TCE, it is unclear if the exposure level was adequate to show results relevant to mortality as there were no effects at the single concentration tested.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substances
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source, strain, or sex of the test guinea pigs were not reported.

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Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate
	Metric 15:	Number per Group	High	× 1	1	20 animals per series
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; mortality was monitored and body weight was recorded
	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	Mortality observations and weight measurements were made for all animals daily except weekends
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	this metric is not rated/applicable because no subjective outcomes were assessed.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were reported; there was no reporting of food/water intake; unlikely 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 for each study group were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Noted that an analysis of variance was applied in the statistical calculations, though statistical tests were not specified. P-values (unspecified significance test) were reported for body weight changes. No statistical significance values were reported for mortality
	Metric 24:	Reporting of Data	Medium	× 2	4	Incidence of mortality was reported for both CCl4 and TCE. Body weight changes was reported for TCE, but not CCl4
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

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Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

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

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

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

Table 4: **Animal toxicity evaluation results of Adams et al., 1952 for an acute inhalation toxicity in rats study on neurological/behavior outcomes**

Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	acute inhalation toxicity in rats
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name and contaminants identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls not required for acute lethality test
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for acute lethality test
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not describe method of animal allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure durations ranged between 0.1 and 12 hours. Acute inhalation lethality tests are typically 4 hours in duration.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	6 exposure groups ranging more than 6-fold (high to low) were used, but the durations of exposure varied by exposure.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic whole body chamber was used for vapor that may condense.
Domain 4: Test Organism						

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	acute inhalation toxicity in rats
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal species, strain, and source (in-house colony) were reported. Study reports using both sexes but does not indicate which sex was used for each exposure level and duration. Initial health status, age, and body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions other than the exposure chamber were not reported.
	Metric 15:	Number per Group	High	× 1	1	Between 5 and 20 animals were used for each combination of concentration and duration. This is more than required for an acute lethality study
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology and outcomes assessed were typical for acute lethality study.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Study reports observing survivors for 2-3 weeks or until full recovery was established. This could lead to inconsistencies in mortality assessment if there are late deaths.
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Mortality is not subjective outcome.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls not required for acute lethality study.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was not conducted, and an LC50 was not identified. Mortality data enabling independent statistical analysis were reported.
	Metric 24:	Reporting of Data	Low	× 2	6	Mortality data are reported, but without time to death and not by sex.
Overall Quality Determination[‡]			~~Medium~~ ⟶ Low[§]		~~2.2~~	
Extracted			Yes			

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	acute inhalation toxicity in rats
HERO ID:	62373

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: "Varying numbers of animals were exposed to different concentrations for different durations and with varying postexposure observation times."

Table 5: **Animal toxicity evaluation results of Kronevi et al., 1979 for an acute dermal study on liver toxicity, kidney toxicity, skin morphology**

Study Citation:	Kronevi, T; Wahlberg, J; Holmberg, B (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs Environmental Research, 19(1,1), 56-69
Data Type:	acute dermal
HERO ID:	3684159

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as carbon tetrachloride (p.a.).
	Metric 2:	Test Substance Source	Medium	× 1	2	Obtained from E. Merck, Darmstadt, Germany. No batch/lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	Not specified, but reported "p.a.", which indicates analytical grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	No control animals were used. Study authors note that skin morphology in exposed area was compared to skin morphology from unexposed area in the same animal. No comparator for liver or kidney histology.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	Study authors did not report animal allocation methods.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Pure solvent was applied, so no preparation was required.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	All animals similarly exposed
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	1 mL of pure solvent applied within a glass ring with an inside diameter of 20 mm (area 3.1 cm2). At a density of 1.59 g/cm3 = 1.59 g/mL, the administered dose was 1.59 g. Glass ring was covered with glass (occluded conditions).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure was for 15 minutes, 1 hr, 4 hr, or 16 hr
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose group (pure solvent), but for 4 durations.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dermal exposure using a covered glass ring to prevent volatilization or exposure via inhalation or oral routes.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Albino guinea pigs weighting between 440 and 570 g. Source and sex of animals not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	No husbandry conditions were reported, but since this is an acute study this is not likely to have a major impact on study.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per group were not explicitly reported. Overall number of animals was 20. There were 5 compounds tested, with each compound evaluated for 4 time-points. This implies that only one animal was used per compound per duration.

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Study Citation:	Kronevi, T; Wahlberg, J; Holmberg, B (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs Environmental Research, 19(1,1), 56-69
Data Type:	acute dermal
HERO ID:	3684159

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Skin biopsy was performed on exposed area and neighboring unexposed animal. Liver and kidney histology were assessed for evaluation of liver and kidney histology.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Only one animal per group and no controls, so sampling adequacy is N/A
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not required for initial histopathological review.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	Skin biopsy results from untreated skin were not reported. No control specimens for liver or kidney histology.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Little concern with confounding in acute study design
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	Attrition/infection N/A due to acute study design
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	No statistical methods. Only one animal per group, so data insufficient for statistical analysis.
	Metric 24:	Reporting of Data	Medium	× 2	4	Results reported qualitatively.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 6: **Animal toxicity evaluation results of Adams et al., 1952 for an acute inhalation toxicity in rats study on mortality outcomes**

Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	acute inhalation toxicity in rats
HERO ID:	62373

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name and contaminants identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	Negative controls not required for acute lethality test, but neurotoxicity cannot be assessed without negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for acute lethality test
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not describe method of animal allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure durations ranged between 0.1 and 12 hours.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	6 exposure groups ranging more than 6-fold (high to low) were used, but the durations of exposure varied by exposure.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic whole body chamber was used for vapor that may condense.
Domain 4: Test Organism						

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	acute inhalation toxicity in rats
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal species, strain, and source (in-house colony) were reported. Study reports using both sexes but does not indicate which sex was used for each exposure level and duration. Initial health status, age, and body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions other than the exposure chamber were not reported.
	Metric 15:	Number per Group	High	× 1	1	Between 5 and 20 animals were used for each combination of concentration and duration. This should be adequate for acute toxicity
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Frequency and timing of observation for clinical signs of neurotoxicity was not described.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	No information on consistency of clinical observations was provided.
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Unacceptable	× 1	4	Observations for clinical signs may be subjective and blinding was not reported.
	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/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not conducted, and data enabling independent statistical analysis were not reported.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Incidences of clinical signs of neurotoxicity were not reported
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.6	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

[⋆] MWF = Metric Weighting Factor.

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

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

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

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

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

2 Short-term (1-30 days)

Table 7: **Animal toxicity evaluation results of Hayes et al., 1986 for an 14 day oral toxicity test in mice study on mortality, clinical chemistry/ biochemical , renal, hepatic, respiratory, hematological and immune, neurological/behavior, and reproductive outcomes**

Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	14 day oral toxicity test in mice
HERO ID:	194400

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	Medium	× 1	2	Test substance source and lot number reported, but certification/analytical verification of identity was not.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance reported to be HPLC grade and >99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Both naive and sham-treated control groups were used. Sham-treated controls received vehicle.
	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 randomizing the mice but does not discuss the allocation to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Study reports daily preparation of solution, but does not report storage.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Administration details are provided, including gavage volume and time of day of administration. No inconsistencies in exposures across groups were noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose reported in mg/kg bw; body weight not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals gavaged daily for 14 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	3 nonzero doses ranging 4-fold were used. Effects were seen at all doses, so it is not clear that the lowest dose was low enough.
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	20/sex/dose were tested; this is more than adequate.
Domain 5: Outcome Assessment						

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Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	14 day oral toxicity test in mice
HERO ID:	194400

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was described in detail and appropriate. Histopathology was not evaluated, but organ weights, serum chemistry, and hematology were. Food and water intake were not reported. The only neurological and reproductive endpoints assessed were brain and testes weights, respectively.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were noted by the authors apart from one gavage death in high dose females.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Mortality, organ weights evaluated in all animals; hematology and serum chemistry evaluated in 5/sex/dose each.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were evaluated
	Metric 20:	Negative Control Response	High	× 1	1	Responses of both naive and vehicle controls were reported for all endpoints other than hematology. Responses appeared to be as expected and without excessive variability.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	The study reports that 20 animals/sex/dose were tested, and that organ weights were evaluated in all animals; however, results are reported for only 10 animals/sex/dose. The study authors do not explain this discrepancy.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate to the data.
	Metric 24:	Reporting of Data	Low	× 2	6	Mortality, organ weights, and significant clinical chemistry findings were reported; body weight, hematology results, and non-significant clinical chemistry findings were not. The lack of body weight data is problematic for interpretation of relative organ weight changes.
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 8: **Animal toxicity evaluation results of Narotsky et al., 1997 study**

Study Citation:	Narotsky, MG; Pegram, RA; Kavlock, RJ (1997). Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats Fundamental and Applied Toxicology, 40(1), 30-36					
Data Type:						
HERO ID:	194607					
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	High	× 2	2	
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group is not required for study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Method for allocation "assured a homogeneous distribution of body weights among groups".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Information on stability of dosing formulations is not provided and frequency of preparation is not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Animals were exposed only on GD 6-15. More complete information on developmental effects of CCl4 could be determined if animals were exposed through the entire gestation period.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Age of animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	12-13 dams per group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Main focus of study was full-litter resorption.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						

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Study Citation:	Narotsky, MG; Pegram, RA; Kavlock, RJ (1997). Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats Fundamental and Applied Toxicology, 40(1), 30-36
Data Type:	
HERO ID:	194607

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	
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 9: **Animal toxicity evaluation results of Benson et al., 1999 for a 4-week oral (rats, mice, hamsters) study on clinical chemistry/biochemical, and hepatic outcomes**

Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report					
Data Type:	4-week oral (rats, mice, hamsters)					
HERO ID:	195107					
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	Low	× 1	3	The source and batch/lot number of the test substance was not reported. The omitted details are likely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade of the test substance were not reported and this may have a substantial impact on the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using a concurrent negative control group but details regarding the negative control group were not reported and the lack of details may have a substantial impact on the results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not indicated for the study type.
	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	Low	× 1	3	The study authors did not describe the test substance preparation and storage conditions. The reporting deficiencies are likely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and the exposures were administered consistently across study groups (exposed in drinking water ad libitum).
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Target concentrations in drinking water were reported; however, deficiencies in reporting of drinking water intakes and resulting actual exposures are likely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (ad libitum in drinking water, 7 days/week, 1 or 4 weeks) were reported and acceptable for the outcomes of interest (clinical chemistry, liver histopathology, liver proliferation via BrdU labeling [mechanistic]. However, it should be noted that the exposure of up to 4 weeks, with no effects on liver histopathology, contrast with the 12-week inhalation regimen reported in the same reference (reviewed in a separate form), which induced liver histopathology.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	There were minor limitations regarding the doses selected (0, 500, 5000 ppb), as it is not evident that the highest dose was high enough (no adverse effects reported).

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Study Citation: Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type: 4-week oral (rats, mice, hamsters)
HERO ID: 195107

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The species, strain, and sex of the animals were reported; however, source, health status, age, and starting body weight were not reported.
	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 populations.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (5 or 6 per time point of sacrifice) was less than typically used in studies of the same or similar type (i.e., subchronic-type studies).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive for the outcomes of interest (primarily hepatic and clinical chemistry outcomes, with mechanistic liver evaluation).
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details regarding the execution of the study protocol for outcome assessment, including time of assessments across study groups, were not fully reported. These reporting deficiencies may have a substantial impact on the results.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported, which may have a substantial impact on the results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The negative control responses were reported and acceptable.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control responses were reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and water intake were not reported and this may have a substantial impact on results because it was not reported if there were any palatability issues.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and health outcomes unrelated to exposure were not reported for each study group and this deficiency may have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were not described clearly and this deficiency is likely to have a substantial impact on results.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not clearly shown for each study group; however, results were briefly described in the text. This may have a substantial impact on the results.
Overall Quality Determination[‡]			Low		2.3	
Extracted			Yes			

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Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type:	4-week oral (rats, mice, hamsters)
HERO ID:	195107

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 10: **Animal toxicity evaluation results of Sun et al., 2014 for a study on hepatic outcomes**

Study Citation:	Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics Metabolomics, 10(6), 1293-1304
Data Type:	
HERO ID:	3487830

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	Commercial source was identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle (corn oil) controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not used for liver toxicity.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to each dose group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage were not described; however, omission of these details are unlikely to have a substantial impact on results (acute exposure).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Acute studies are included; liver effects occur after acute exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Two dose groups plus control. High dose chosen to induce mild to moderate adverse effects based on range-finding study. 30 animals received single dose and an additional 15 animals received a total of 3 once daily doses, which should be sufficient for the main purpose of this study (e.g., metabolomics).
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	FDA colony; species, strain and starting age reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	30 animals received single dose and an additional 15 animals received a total of 3 once daily doses
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Clinical chemistry and liver histopathology.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Medium	× 1	2	5/group used for clinical chemistry and histopathology.

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Study Citation:	Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics Metabolomics, 10(6), 1293-1304
Data Type:	
HERO ID:	3487830

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were well-described.
	Metric 24:	Reporting of Data	High	× 2	2	
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 11: **Animal toxicity evaluation results of Civo et al., 1985 for a 4 week inhalation-liver toxicity (same as 4215910) study on hepatic outcomes**

Study Citation:	Civo Institute Tno (1985). Fixed versus variable levels of exposure in inhalation toxicity testing with reference to the workplace studies with acetaltehyde and carbon tetrachloride
Data Type:	4 week inhalation-liver toxicity (same as 4215910)
HERO ID:	4215798

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source was reported incompletely, but the omitted details are unlikely to have a substantial impact on results
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Randomized Allocation	High	× 1	1	Computer randomization used for allocation.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment of generation was reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	the number of groups and concentration spacing were reported and justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were reported and appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, species, strain, sex, and initial body weight were reported. Health status and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry details were reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						

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Study Citation:	Civo Institute Tno (1985). Fixed versus variable levels of exposure in inhalation toxicity testing with reference to the workplace studies with acetaltehyde and carbon tetrachloride
Data Type:	4 week inhalation-liver toxicity (same as 4215910)
HERO ID:	4215798

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Respiratory rate was not reported but is not likely to have significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	
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.

3 Other

Table 12: **Animal toxicity evaluation results of Benson et al., 1999 for a inhalation and ADME studies (acute and subchronic) study on ADME/ PBPK outcomes**

Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report					
Data Type:	inhalation and dw ADME studies (acute and subchronic)					
HERO ID:	195107					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source of radiolabeled CCL4 was given, but not lot number.
	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	Negative controls were used in inhalation and dw studies of CYP expression and activity. Controls were not necessary for inhalation studies of uptake, distribution and clearance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not needed for ADME studies.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Radiolabeled solutions were prepared immediately prior to exposure. For inhalation studies, the method and equipment used to generate the test substance as a vapor, was reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Actual concentrations were not reported for inhalation experiments.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Continuous exposure in dw for 1 or 4 weeks; 6h/day, 5 days a week for 1 or 12 weeks via inhalation .
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Dose groups and spacing were not justified; however, dose response relationsips were evident (e.g., CYP protein levels).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Inhalation rout and method are appropriate (nose-only and whole-body). It is unclear whether CCL4 is fully soluble in dw at the concentration used (not addressed in the study).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Mutiple species were obtained from a commercial source and starting bw were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and adequate.
	Metric 15:	Number per Group		× 1	NA	20 animals/species/group (all males).

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Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type:	inhalation and dw ADME studies (acute and subchronic)
HERO ID:	195107

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	Studies of uptake, distribution and clearance in multiple species. CYP induction following in vivo exposure.
	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 outcomes were objective.
	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	respiratory rate was not reported and CCL4 is expected to be a respiratory irritant.
	Metric 22: Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	Not applicable to ADME data.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	
	Metric 24: Reporting of Data	High	× 2	2	ADME data were fully reported in data tables.
Overall Quality Determination[‡]		High		0.0	
Extracted		No			

⋆ MWF = Metric Weighting Factor.

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

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

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

where High $=\geq 1$ 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 Adams et al., 1952 for a subchronic inhalation exposures (46 to 94 days) in rats study on renal, hepatic, nutrition and metabolic/adult exposure body weight, and cardiovascular outcomes**

Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	subchronic inhalation exposures (46 to 94 days) in rats
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name and contaminants identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Both untreated and sham-treated control groups were used.
	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 describe method of animal allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method.
	Metric 10:	Exposure Frequency and Duration	Unacceptable	× 1	4	Exposure frequencies ranged between 0.05 and 1 hour per day. This well below the daily duration typically used for subchronic toxicity evaluation.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure concentrations were tested; the low concentration was tested at four different daily exposure durations (0.05 to 1 hr/day)

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Study Citation: Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type: subchronic inhalation exposures (46 to 94 days) in rats
HERO ID: 62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Medium	× 1	2	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, and source (in-house colony) were reported. Study reports choosing animals for the study based on health during pre-exposure observation period. Age and initial body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions other than the exposure chamber were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Group sizes were 5 or 6/sex/group. This is consistent with recommendations for 28 day studies but less than recommended for subchronic studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described, and outcomes included body weight and weights and histopathology of liver, kidney, lung, and heart.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in assessment of these endpoints were reported.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were evaluated for these endpoints.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were evaluated.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	Control responses were not reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was conducted and methodology described; the method did not account for multiple comparisons.
	Metric 24:	Reporting of Data	Low	× 2	6	All data were reported qualitatively without indication of which control group(s) was compared for statistical analysis.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.1	
Extracted			No			

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	subchronic inhalation exposures (46 to 94 days) in rats
HERO ID:	62373

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 Bruckner et al., 1986 for a study on renal and hepatic outcomes**

Study Citation:	Bruckner, JV; Mackenzie, WF; Muralidhara, S; Luthra, R; Kyle, GM; Acosta, D (1986). Oral toxicity of carbon tetrachloride: Acute, subacute, and subchronic studies in rats Fundamental and Applied Toxicology, 6(1), 16-34					
Data Type:						
HERO ID:	62379					
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 (CASRN not provided).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (analytical grade CCl4) was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The grade, but not the purity of the test substance was reported. Since the test substance was obtained from a manufacturer, it is unlikely that impurities would have a substantial impact on the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate control groups were used. Treated animals were administered CCl4 in corn oil via gavage. Control animals were treated with corn oil only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group is not indicated by study type (acute, subacute, and subchronic-duration animal toxicity studies).
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that rats were randomly divided into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicated that CCl4 was mixed with corn oil and administered via gavage in a total volume of 1 mL/animal. Test substance stability/storage conditions were not reported, but are not likely to substantially impact the results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across groups (same frequency, same time of day, consistent gavage volumes).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	The exposure frequency and duration were clearly reported. However, minor limitations in the frequency/duration of treatment were identified (acute, subacute, and subchronic durations were not "standard"). Animals treated a single time by gavage were sacrificed 24 hours after exposure (not followed for up to 14 days); animals treated sub-acutely were administered CCl4 on a cycle of 5 days on, 2 days off, 4 days on (with sacrifice after 4 or 11 days), and animals treated for a subchronic duration were administered CCl4 for 12 weeks (less than 90 days).

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Study Citation: Bruckner, JV; Mackenzie, WF; Muralidhara, S; Luthra, R; Kyle, GM; Acosta, D (1986). Oral toxicity of carbon tetrachloride: Acute, subacute, and subchronic studies in rats Fundamental and Applied Toxicology, 6(1), 16-34

Data Type:

HERO ID: 62379

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of dose groups and dose spacing were justified by the authors and considered adequate to address the purpose of the study (identifying NOAEL and LOAEL levels, with a focus on liver effects).
	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 species, strain, sex, and starting body weights (within a range) were reported.; the species and strain were appropriate. Animals were obtained from a commercial laboratory. Rats were described as adults (specific age not reported). Health status of the rats was not explicitly specified. These minor limitations are unlikely to substantially impact the study results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some husbandry conditions were specified (i.e. reverse light/dark conditions were reported) . The lack of information on other conditions (i.e. temperature, humidity) are considered minor uncertainties that are unlikely to have a substantial impact on the results (no indication that conditions were different among treated rats and controls).
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported.. However, only male rats were used; the number of animals used were 5 for acute and sub-acute studies, and 15-16 for the subchronic-duration study (compared to 10/sex/group used for standard 28-day and 90-day repeated-dose studies).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment partially addressed the intended outcomes of interest. Liver and kidney effects were evaluated by examining limited clinical chemistry parameters, organ weights, and/or histopathological effects. Other common clinical chemistry parameters associated with liver and kidney function were not measured.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocols were described, and outcomes were assessed consistently across groups.Data for liver lesions were presented quantitatively as means (+/-SD) based on severity scores ranging from 0 to 8.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Details regarding sampling for the outcomes of interest were reported, with minor limitations (e.g. number of histology slides evaluated not reported). Numbers of animals evaluated for specific endpoints were generally limited (5-9 males/group, even for the subchronic-duration study).
	Metric 19:	Blinding of Assessors	High	× 1	1	Histopathology slides were coded and examined in a single blind fashion.

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Study Citation:	Bruckner, JV; Mackenzie, WF; Muralidhara, S; Luthra, R; Kyle, GM; Acosta, D (1986). Oral toxicity of carbon tetrachloride: Acute, subacute, and subchronic studies in rats Fundamental and Applied Toxicology, 6(1), 16-34
Data Type:	
HERO ID:	62379

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the control group were reported for most endpoints; however, liver histopathology data for control animals subjected to acute and sub-acute treatment were not shown (no effects were reported). Liver weight data were provided in the text for the control and high-dose groups only.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences in initial body weights among study groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	
	Metric 24:	Reporting of Data	Medium	× 2	4	Data were presented by exposure group for most endpoints. Liver histopathology data for the negative control group (acute and subacute studies) are not shown in the data tables. Liver weight data are provided for the control and high-dose groups only.
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 15: **Animal toxicity evaluation results of Hayes et al., 1986 for 90 day oral toxicity test in mice study on reproductive, hematological and immune, neurological, renal, hepatic, clinical chemistry/biochemical, mortality, nutrition and metabolic/adult exposure body weight, and respiratory outcomes**

Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-l mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	90 day oral toxicity test in mice
HERO ID:	194400

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	Medium	× 1	2	Test substance source and lot number reported, but certification/analytical verification of identity was not.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance reported to be HPLC grade and >99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Both naive and sham-treated control groups were used. Sham-treated controls received vehicle.
	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 randomizing the mice but does not discuss the allocation to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Study reports daily preparation of solution, but does not report storage.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Administration details are provided, including gavage volume and time of day of administration. No inconsistencies in exposures across groups were noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose reported in mg/kg bw; initial body weight not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals gavaged daily for 90 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	4 nonzero doses ranging 100-fold were used. Effects were seen at all doses, so it is not clear that the lowest dose was low enough.
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	20/sex/dose were tested
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Only liver and kidney were examined microscopically
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were noted by the authors.

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Study Citation:	Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice Fundamental and Applied Toxicology, 7(3), 454-463
Data Type:	90 day oral toxicity test in mice
HERO ID:	194400

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Mortality, body weight, histopathology, and organ weights were reportedly evaluated in all animals; hematology and serum chemistry evaluated in subgroups of 8-10/sex/dose due to low blood volume of mice.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Although study reports observing animals for signs of intoxication, no results were reported, so lack of blinding would not be of concern. No other subjective outcomes were evaluated
	Metric 20:	Negative Control Response	High	× 1	1	Responses of both naive and vehicle controls were reported for all endpoints other than hematology. Responses appeared to be as expected and without excessive variability.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Study reports that there were no compound-related deaths, but there were several mortalities in the vehicle control and high dose groups (in males) and in vehicle control and all exposure groups (in females); these were presumably gavage errors.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate to the data.
	Metric 24:	Reporting of Data	Low	× 2	6	There are unexplained inconsistencies in the numbers of animals exposed and evaluated for histopathology vs the numbers of animals for which histopathology results are reported. Although the authors report that histopathology was evaluated in all control and exposed mice, results are reported for only 10/sex/dose (vs 20/sex/dose exposed and evaluated for organ weights). The authors do not explain this apparent discrepancy.
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 16: **Animal toxicity evaluation results of Allis et al., 1990 for a 12-week oral study on hepatic, nutrition and metabolic/adult exposure body weight, and clinical chemistry/biochemical outcomes**

Study Citation:	Allis, JW; Ward, TR; Seely, JC; Simmons, JE (1990). Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats Fundamental and Applied Toxicology, 15(3), 558-570
Data Type:	12-week oral
HERO ID:	194565

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity and grade were not reported and there was no analysis conducted for measurement of impurities, if present.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used and was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not indicated by the study type.
	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	Low	× 1	3	The study authors stated that new gavage solutions were used daily and were prepared weekly; however, the procedures for preparing the solutions in vehicle (corn oil) were incompletely reported and storage conditions were not reported. Deficiencies in reporting may have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details on exposure administration were reported, including consistent dosing volumes, and exposures were administered consistently across study groups in a scientifically sound manner (dose volume of 2 mL/kg was acceptable).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and were appropriate for the study type and outcomes of interest. In this subchronic study, animals were gavaged 5 days per week for 12 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Although two quantitative dose groups (20 and 40 mg/kg/day) were used, there were deficiencies in the dose spacing. Adverse effects, including liver histopathology, clinical chemistry, and reduced body weight gain, were observed at both doses and, in some cases, there were few differences between the two dose groups (e.g., histopathology incidence).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported (gavage) and suited to the test substance.

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Study Citation: Allis, JW; Ward, TR; Seely, JC; Simmons, JE (1990). Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats Fundamental and Applied Toxicology, 15(3), 558-570
Data Type: 12-week oral
HERO ID: 194565

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were reported (source, species, strain, sex, age, starting body weight); however, health status at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported and were adequate and similar for all groups.
	Metric 15:	Number per Group	Medium	× 1	2	Groups were subdivided for some evaluations, resulting in a lower number of animals per group than is typical. For example, from the 24 animals/dose level, 6 animals each were terminated on respective days 1, 8, 15, and 22 post-exposure for evaluation of hepatic cytochrome P450, serum chemistry, and light microscope histopathology, resulting in only 6 animals/dose group evaluated for these endpoints.
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, which were primarily effects on the liver.
	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	Medium	× 1	2	Details regarding sampling for the outcomes of interest were reported and were adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported. Blood samples were assayed commercially and histopathology was not described as a re-evaluation so I scored this metric as not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	The 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 reported differences among the study groups in initial body weight or food or water intake that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and 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						

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Study Citation:	Allis, JW; Ward, TR; Seely, JC; Simmons, JE (1990). Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats Fundamental and Applied Toxicology, 15(3), 558-570
Data Type:	12-week oral
HERO ID:	194565

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analyses that were conducted were not described clearly for each endpoint evaluated. Statistical analyses/results were not reported for the hepatic histopathology data (Table 2 of the study report); however, sufficient data were provided to allow an independent analysis. Statistical analysis results were not shown for body weights. Although body weight gain data were provided in a figure (Figure 6), the data were provided without mean values and error bars.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented by exposure group, with quantal and/or continuous presentation, as well as severity scores. Negative findings were reported in the text.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "I would downgrade the study to medium for the follow reasons: lack of details on test substance purity/grade and lack of details on preparation of test substance and storage, both in the absence of reported measurement of test solutions demonstrating stability of test substance in the prepared solutions during the one week storage period and under the conditions of storage, given potential volatility of CCl4."

Table 17: **Animal toxicity evaluation results of Condie et al., 1986 for a 90-day oral study on mortality, metabolic/adult exposure body weight, hepatic, and clinical chemistry/biochemical outcomes**

Study Citation:	Condie, LW; Laurie, RD; Mills, T; Robinson, M; Bercz, JP (1986). Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-1 mice: corn oil versus Tween-60 aqueous emulsion Toxicological Sciences, 7(2), 199-206
Data Type:	90-day oral
HERO ID:	60712

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance, including manufacturer and lot number, was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity was reported (98.2%) and impurities (chloroform, 1.8%) were identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using appropriate concurrent negative control groups (corn oil and Tween-60).
	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 authors 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	The study authors did not report preparation and storage conditions, including how often test substance was prepared and under what conditions the test substance was stored. Deficiencies in reporting may have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and spacing were reported and considered adequate for the purpose of the study. Selected concentrations were not justified by the study authors but the selected doses appear acceptable.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and these were suited to the test substance.
Domain 4: Test Organism						

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Study Citation: Condie, LW; Laurie, RD; Mills, T; Robinson, M; Bercz, JP (1986). Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-1 mice: corn oil versus Tween-60 aqueous emulsion Toxicological Sciences, 7(2), 199-206
Data Type: 90-day oral
HERO ID: 60712

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The animal species, strain, and sex were reported; however, age, starting body weight, and health status were not reported. The test species was obtained from a commercial source and was an appropriate model for evaluation of the outcomes of interest. The reporting deficiencies are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate and the same for control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment protocol was reported; however, the descriptions of sampling of blood for serum enzymes do not clearly indicate when blood was collected from the animals.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported by the study authors and the study used adequate sampling for the outcomes of interest (e.g., adequate number of animals from each group).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The study did not report evaluation of subjective outcomes except histopathology. According to the criteria, however, this metric is not rated/applicable for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The 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 among the study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition or health outcomes unrelated to exposure 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 and appropriate for the data set.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex with quantal and/or continuous presentation and description of severity scores.

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Study Citation:	Condie, LW; Laurie, RD; Mills, T; Robinson, M; Bercz, JP (1986). Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-1 mice: corn oil versus Tween-60 aqueous emulsion Toxicological Sciences, 7(2), 199-206
Data Type:	90-day oral
HERO ID:	60712

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
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 18: **Animal toxicity evaluation results of Benson et al., 1999 for a 12-week inhalation (rats, mice, hamsters) study on clinical chemistry/ biochemical, and hepatic outcomes**

Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report					
Data Type:	12-week inhalation (rats, mice, hamsters)					
HERO ID:	195107					
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	Low	× 1	3	The source and batch/lot number of the test substance was not reported. The omitted details are likely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade of the test substance were not reported and this may have a substantial impact on the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using a concurrent negative control group but details regarding the negative control group were not reported and the lack of details may have a substantial impact on the results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not indicated for the study type.
	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	Low	× 1	3	The study authors did not describe the test substance preparation and storage conditions. The reporting deficiencies are likely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details, including the methods for generating atmosphere in inhalation chambers, were not reported.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Target concentrations were reported; however, actual concentrations were not reported and there was no indicated that test concentrations were monitored/measured during the inhalation exposures.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration and frequency (6 hours/day, 5 days/week, for 1, 4, or 12 weeks) were reported and were suited to the study type and outcomes of interest (clinical chemistry, liver histopathology, and liver proliferation/mechanistic [via BrdU labeling]).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing (0, 5, 20, 100 ppm) were reported and were relevant for the assessment. Although not justified, it appears that concentrations were based on results reported in previous studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.

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Study Citation: Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type: 12-week inhalation (rats, mice, hamsters)
HERO ID: 195107

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 4: Test Organism					
Metric 13:	Test Animal Characteristics	Low	× 2	6	The species, strain, and sex of the animals were reported; however, source, health status, age, and starting body weight were not reported.
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 populations.
Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (5 or 6 per time point of sacrifice) was less than typically used in studies of the same or similar type (i.e., subchronic-type studies).
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive for the outcomes of interest (primarily hepatic and clinical chemistry outcomes, with mechanistic liver evaluation).
Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details regarding the execution of the study protocol for outcome assessment, including time of assessments across study groups, were not fully reported. These reporting deficiencies may have a substantial impact on the results.
Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported, which may have a substantial impact on the results.
Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
Metric 20:	Negative Control Response	High	× 1	1	The negative control responses were reported and acceptable.
Domain 6: Confounding / Variable Control					
Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate measurement was not reported. This is considered to have a substantial impact on results since CCl4 is a potential respiratory irritant.
Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and health outcomes unrelated to exposure were not reported for each study group and this deficiency may have a substantial impact on results.
Domain 7: Data Presentation and Analysis					
Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were not described clearly and this deficiency is likely to have a substantial impact on results.
Metric 24:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not clearly shown for each study group; however, results were briefly described in the text. This may have a substantial impact on the results.
Overall Quality Determination[‡]		~~Unacceptable~~[⋆⋆] ⟶ Low[§]		~~2.4~~	
Extracted		Yes			

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Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type:	12-week inhalation (rats, mice, hamsters)
HERO ID:	195107

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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.

[§] Evaluator's explanation for rating change: "The study may inform on liver outcomes although it is not likely to be useful for dose-response assessment."

Table 19: **Animal toxicity evaluation results of Benson et al., 1999 for an inhalation and drinking water ingestion studies (1, 4 and 12 weeks) study on hepatic outcomes**

Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report					
Data Type:	Inhalation and dw ingestion studies (1, 4 and 12 weeks)					
HERO ID:	195107					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	Low	× 1	3	No details were provided on them source of the test substance.
	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	Negative air and dw controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not generally included in studies of liver toxicity.
	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	Inhalation exposure details were provided in the ADME study description (method of vapor generation was described and appropriate).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Actual concentrations were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Continuous exposure for dw; 6hour/day 5 days/wk, for 1, 4 or 12 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2-3 groups plus control; doses were not justified , but dose response relationships were apparent.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic whole-body chambers.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Rat, mouse and hamster species, strain and age were reported in the ADME study, Obtained from commercial source.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Adequate husbandry conditions as described in ADME studies.
	Metric 15:	Number per Group	Medium	× 1	2	5-6/group for most endpoints (10/group for serum chemistry at 12 weeks); adequate for statistics.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome methods were sensitive for hepatotoxicity (serum chemistry, histopath. and hepatocellular replication).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	

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Study Citation:	Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
Data Type:	Inhalation and dw ingestion studies (1, 4 and 12 weeks)
HERO ID:	195107

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported ; however, outcomes were objective.
	Metric 20:	Negative Control Response	High	× 1	1	No incidence of hepatocellular necrosis in controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not measured; CCl4 is anticipated to be a respiratory irritant.
	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	
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all time points and exposure groups.
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.

5 Chronic (>90 days)

Table 20: **Animal toxicity evaluation results of Nagano et al., 2007 for a 2-year bioassay study on cancer, mortality, hepatic, renal, respiratory, endocrine, clinical chemistry/biochemical, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	Nagano, K; Sasaki, T; Umeda, Y; Nishizawa, T; Ikawa, N; Ohbayashi, H; Arito, H; Yamamato, S; Fukushima S (2007). Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice Inhalation Toxicology, 19(13), 1089-1103
Data Type:	2-year bioassay
HERO ID:	194127

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer; however, the batch/lot number was not reported, although identity was verified by analytical means (gas chromatography) by the study laboratory.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity and composition were such that any observed effects were highly likely to be due to the test substance itself. The purity was reported as 99.8% and other components were identified with purities provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control group is not indicated by study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	The study reported that the animals were divided by stratified randomization; however, there were minor limitations in the allocation method due to use of a non-random component (body weight-matched grouping).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation methods were reported and appropriate for the test substance. The method and equipment for generating the test substance as a vapor were reported and appropriate. Although not reported, test substance storage conditions were considered appropriate based on observation of stability of the test substance before and after use by gas chromatography and infrared spectrometry analysis.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure methods were reported and exposures were administered consistently for the study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity. Mean measured concentrations were reported based on chamber concentrations monitored every 15 minutes during the exposures by gas chromatography.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and were appropriate for the study type and outcomes of interest.

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Study Citation: Nagano, K; Sasaki, T; Umeda, Y; Nishizawa, T; Ikawa, N; Ohbayashi, H; Arito, H; Yamamato, S; Fukushima S (2007). Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice Inhalation Toxicology, 19(13), 1089-1103
Data Type: 2-year bioassay
HERO ID: 194127

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and concentration spacing were justified; however, the highest concentration (125 ppm) resulted in early mortality of most animals. Therefore, there were an insufficient number of animals in this group for statistical analysis of some endpoints, including terminal body weights, organ weights, clinical chemistry, and urinalysis. Two lower concentrations, 5 and 25 ppm, were also included in the study and a sufficient number of animals survived the duration of exposure for statistical analysis on the same endpoints.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and 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 was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported, including temperature, humidity, and light-dark cycle, and were adequate and no differences were reported for the test substance-exposed and control groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group (50/sex/group) was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed 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 using the same protocol.
	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 so I considered this metric not applicable. Blood samples were analyzed automatically and histopathology was not described as a re-evaluation.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were no confounding differences among the study groups in initial body weight. Respiratory rate, however, was not reported.

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Study Citation:	Nagano, K; Sasaki, T; Umeda, Y; Nishizawa, T; Ikawa, N; Ohbayashi, H; Arito, H; Yamamato, S; Fukushima S (2007). Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice Inhalation Toxicology, 19(13), 1089-1103
Data Type:	2-year bioassay
HERO ID:	194127

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 study groups were noted.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	The statistical methods were clearly described and appropriate for the data.
	Metric 24: Reporting of Data	High	× 2	2	
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 21: **Animal toxicity evaluation results of Nagano et al., 2007 for a 13-week inhalation study in rats and mice study on renal, hepatic, hematological and immune, clinical chemistry/biochemical , and body weight outcomes**

Study Citation:	Nagano, K; Umeda, Y; Saito, M; Nishizawa, T; Ikawa, N; Arito, H; Yamamoto, S; Fukushima, S (2007). Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice Journal of Occupational Health, 49(4), 249-259
Data Type:	13-week inhalation study in rats and mice
HERO ID:	194237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Analytical-grade CCl4
	Metric 2:	Test Substance Source	High	× 1	1	source clearly identified..
	Metric 3:	Test Substance Purity	High	× 1	1	purity specified (98%); each lot analyzed for stability and purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	used appropriate concurrent negative control group (clean air) under the same conditions as treated groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	this metric is not rated/ applicable because a positive control is not indicated by this study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Animals allocated using stratified randomization into weight-matched 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.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were clearly reported and were consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal was obtained through the reported commercial source. The test animal species, strain, sex, and age were specified. Starting body weight was not reported, but the authors note that animals were randomized into weight-matched groups. The authors also don't explicitly mention health status of the animals. These omissions are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and adequate. Conditions were the same for control and treated groups.

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Study Citation:	Nagano, K; Umeda, Y; Saito, M; Nishizawa, T; Ikawa, N; Arito, H; Yamamoto, S; Fukushima, S (2007). Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice Journal of Occupational Health, 49(4), 249-259
Data Type:	13-week inhalation study in rats and mice
HERO ID:	194237

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended 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 adequate. Endpoints were evaluated in an adequate number of animals in each group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Most outcomes were not subjective; this metric is not rated/applicable for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There was a lack of reporting of respiratory rates; but this lack of reporting is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcome unrelated to exposure for each study group were not reported; the lack of reporting 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 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 and sex
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 22: **Animal toxicity evaluation results of Adams et al., 1952 for a 6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys study on renal, hepatic, respiratory, cardiovascular, hematological and immune, nutrition and nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name and contaminants identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Both untreated and sham-treated control groups were used.
	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 describe method of animal allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposures were 7 hr/day, 5 d/wk for durations ranging up to about 6 months.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	7 exposure concentrations were tested in rats and guinea pigs, with an overall range of 80-fold. 5 concentrations with a range of 20 fold were tested in rabbits and monkeys. Concentrations were sufficient to identify effect levels.

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys
HERO ID:	62373

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Medium	× 1	2	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, and source (in-house colony) were reported. Study reports choosing animals for the study based on health during pre-exposure observation period. Age and initial body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions other than the exposure chamber were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Group sizes were 15/sex for rats, 8/sex for guinea pigs, 2/sex for rabbits, and 2 monkeys.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described, and outcomes included body weight and weights and histopathology of liver, kidney, lung, heart, and spleen. Authors note that limited blood chemistry and hematology endpoints were assessed "in many cases".
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Authors note that limited blood chemistry, liver lipids, and hematology endpoints were assessed "in many cases" but do not specify which groups were evaluated.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding outcome sampling were not reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Endpoints were not subjective.
	Metric 20:	Negative Control Response	Low	× 1	3	Control responses were reported only for body weights and organ weights.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was conducted and methodology described; the method did not account for multiple comparisons.
	Metric 24:	Reporting of Data	Low	× 2	6	All data were reported qualitatively without indication of which control group(s) was compared for statistical analysis.
Overall Quality Determination[‡]			~~Medium~~ ⟶ Low[§]		~~2.1~~	
Extracted			Yes			

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Study Citation:	Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
Data Type:	6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys
HERO ID:	62373

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: "Limited (predominantly qualitative) reporting of results, varying exposure durations."

6 Genetic toxicity studies

Table 23: **Animal toxicity evaluation results of Nath et al., 1990 study on DNA adducts**

Study Citation:	R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compunds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357
Data Type:	DNA adducts (32P-postlabeling assay)
HERO ID:	6146

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle controls were used (same injection volume).
	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	Preparation in corn oil was described. Storage was not reported; however, only a single injection was used.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Information was provided to allow calculation of dose (% v/v, ml/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose was adequate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose group; level was not justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported. The test animal was obtained from a commercial source. Body weight and health status were not reported. Mice were described as retired breeders (10-12 months old).
	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	3-4/group was adequate for the outcome of interest.
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	High	× 1	1	Outcome was assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.

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Study Citation:	R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compunds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357
Data Type:	DNA adducts (32P-postlabeling assay)
HERO ID:	6146

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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 appears 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	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 described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were fully reported across timepoints.
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 24: **In vitro evaluation results of Tafazoli et al., 1988 for DNA damage (Comet assay)**

Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	DNA damage (Comet assay)
HERO ID:	194476

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified as Carbon tetrachloride and CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source was reported
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent media-alone controls were used (no solvents were used during test preparation)
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls for conditions with and without metabolic activation were used.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were clearly described and appropriate for the outcome of interest.
	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	Low	× 1	3	Information on test substance preparation was adequately described. Methods were employed (use of sealed bottles) to prevent evaporation during the process. The duration of the test substance preparation however was lengthy (48hours, shaking at 37 degrees), and the rationale for this and the potential impact on stability was not discussed. There is further uncertainty about the stability of the test substance due to lack of DMSO as a solvent.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Consistent application methods are inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Initial Test substance concentrations (3) were reported without ambiguity. Analytical concentrations measured after the media preparation procedure were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest
	Metric 12:	Exposure Route and Method	Low	× 1	3	The concentrations chosen (three) were based on previous experiments evaluating micronuclei. The highest concentration was not cytotoxic in previous experiments and no positive response was observed. Evaluating the highest concentration resulting in some cytotoxicity would be warranted.
	Metric 13:	Metabolic Activation	Low	× 1	3	The study included conditions of metabolic activation (s9), however, the source and method of preparation of the S9 mix were not provided.

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Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	DNA damage (Comet assay)
HERO ID:	194476

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model					
Metric 14:	Test Model	High	× 2	2	The test model (primary human lymphocytes) was appropriate. Descriptive information on the source and method of isolation was provided.
Metric 15:	Number per Group	Medium	× 1	2	Two replicates ("parallel cultures" from each donor) were utilized. This is considered to be somewhat lacking.
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the outcome of interest
Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across exposure groups
Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was generally adequate for the outcome of interest (100 cells total from two cultures).
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 confounding variables in the test design or procedures were identified.
Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables on outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis					
Metric 22:	Data Analysis	High	× 1	1	Statistical methods were clearly stated and appropriate for the outcome of interest.
Metric 23:	Data Interpretation	High	× 2	2	Circumstances yielding a positive result were described. Positive results were based on reaching statistical significance.
Metric 24:	Cytotoxicity Data	Medium	× 1	2	Concurrent cytotoxicity evaluations were not done with this experiment, however cytotoxicity was assessed both in a preliminary range-finding study, and was also evaluated in micronuclei tests that were performed in cells from the same donors using the same concentrations tested for this outcome.
Metric 25:	Reporting of Data	High	× 2	2	Data was adequately presented across all groups
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

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Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	DNA damage (Comet assay)
HERO ID:	194476

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 25: **In vitro evaluation results of Castro et al., 1994 study on thymine binding**

Study Citation:	G. D. Castro, J. T. Simpson, J. A. Castro (1994). Interaction of trichloromethyl free radicals with thymine in a model system: a mass spectrometric study Chemico-Biological Interactions, 90(1,1), 13-22					
Data Type:	Thymine binding					
HERO ID:	194538					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as CCL4, however the main interest was on trichloromethyl (CCL3), a free radial formed during metabolic activation of CCL4 that is expected to bind to nucleic acids.
	Metric 2:	Test Substance Source	High	× 1	1	A commercial source was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Reported as analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Blanks without thymine or benzoyl peroxide were used as negative controls. The study specifically indicated it was not possible to run blanks without CCL4 because it is the only solvent of the reaction mixture.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not necessary for the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were adequately reported. CCL4 was heated in a reaction with thymine and benzoyl peroxide in a sealed system. Reaction products were analyzed by GLC/MS.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The study indicates that CCL4 acted as the solvent for thymine in the reaction. The reaction mixture was purged with nitrogen before sealing in an ampoule.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Multiple groups were not used.
	Metric 10:	Reporting of Doses/Concentrations	Not Rated	NA	NA	No concentration was provided. A 3 mL volume was used.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The reaction time (5hr) was reported
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Not applicable for the study design
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for the study design
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	Not applicable for the study design (chemical reaction, no cells or tissues used)
	Metric 15:	Number per Group	Low	× 1	3	This study does not report replicates, or indicate that the profiles shown were representative of multiple runs.
Domain 5: Outcome Assessment						

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Study Citation:	G. D. Castro, J. T. Simpson, J. A. Castro (1994). Interaction of trichloromethyl free radicals with thymine in a model system: a mass spectrometric study Chemico-Biological Interactions, 90(1,1), 13-22
Data Type:	Thymine binding
HERO ID:	194538

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcomes were assessed using GLC/MS. The study indicated that this was the only technique that could be used due to the low product yield from the reaction mixture, which prevented use of other methods like NMR.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	It is inferred from the text that the same outcome assessment was applied to test and control 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
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Not applicable for the study design
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Not Rated	NA	NA	Not applicable for the study design
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Not applicable for the study design
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Not applicable for the study design - Study design did not involve positive or negative findings, only presence of reaction products.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Not applicable for the study design
	Metric 25:	Reporting of Data	Medium	× 2	4	GLC/MS profiles were included in the report, but profiles from control samples were not provided.
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 26: **In vitro evaluation results of Amacher et al., 1983 study on mammalian cell transformation**

Study Citation:	D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296					
Data Type:	Mammalian cell transformation for CCl4					
HERO ID:	194590					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride.
	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.
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 (ethyl methanesulfonate and benzo[a]pyrene) and appropriate. Positive controls yielded positive results.
	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. Test substance storage was not reported; however, solutions were prepared immediately before administration (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 are reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing was reported and appropriate for this assay.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and method of isolation of the primary Syrian golden hamster embryo cells used here were reported and appropriate for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 30 wells per dose level per test substance.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.

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Study Citation:	D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296
Data Type:	Mammalian cell transformation for CCl4
HERO ID:	194590

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	Low	× 2	6	Initial conditions were not reported for each 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	This metric is not applicable to the study design. Statistical analysis was not conducted on these data; any transformed colonies > 0 was considered a positive result. The raw data do not allow for an independent analysis because the data yielded from multiple doses per test substance were apparently pooled.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and evaluation criteria for assessing transformed colonies were cited to other publications.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	A preliminary toxicity assay was conducted to assess cytotoxicity levels. The doses for the mutagenicity assay were selected so that 50-90% survival was permitted. It is unclear what the methodology for assessing cytotoxicity was, and it is unclear whether cytotoxicity was assessed concurrently with the transformation assay.
	Metric 25:	Reporting of Data		× 2	NA	Raw data yielded from multiple dose levels per test substance were apparently pooled. Therefore, the data reporting is inadequate.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 27: **In vitro evaluation results of Varma et al., 1988 study on Ames assay**

Study Citation:	M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures Journal of Applied Toxicology, 8(4,4), 243-248
Data Type:	Ames assay _CCL4 and chloroform
HERO ID:	194606

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by established nomenclature; no CASRNs were provided
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance source was not identified
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study reports using negative controls, but it is unclear if they were untreated or solvent controls. It was reported however, that separate experiments with the solvent (methanol) were performed and that it was both nontoxic and nonmutagenic.
	Metric 5:	Positive Controls	Low	× 2	6	Appropriate positive controls were included for studies with (2-anthramine) and without (sodium azide, 2-nitrofluorene, and 9-aminoacridine) activation. However, the positive control responses were not reported.
	Metric 6:	Assay Procedures	Medium	× 1	2	The assay procedure was cited to another study with some details provided (plate incorporation assay); assay is standard for evaluating the outcome of interest;
	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	Low	× 1	3	Preparation of test substances was adequately described, however details of mixing, stability and considerations of volatility were not discussed. Plate incorporation method uses; this method does not mitigate volatilization; however, positive results were seen so levels in medium were high enough to induce effect. Test substance storage was not reported
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure administration was not described in detail, studies were performed according to another publication.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations can be estimated from the figures provided.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (2 days) was reported and appropriate for the study design
	Metric 12:	Exposure Route and Method	Low	× 1	3	Six (CCL4) or five (chloroform) concentration groups were tested. No justification for the concentrations tested were provided. It is unknown whether concentrations were adequate or tested up to the level of cytotoxicity.

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Study Citation: M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures Journal of Applied Toxicology, 8(4,4), 243-248
Data Type: Ames assay _CCL4 and chloroform
HERO ID: 194606

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Aroclor-1254 induced rat livers were commercially obtained. 0.5 mL S9 was used for metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (strains of S. typhimurium) are routinely used for the outcome of interest. The source (B.Ames) was reported.
	Metric 15:	Number per Group	Medium	× 1	2	Tests without activation were performed on 4 strains of S. typhimurium. With activation, only two strains were used for CCL4. Generally, these numbers are less than typically recommended number (5) for a mutagenicity study; E. coli was also not included. Each concentration was tested in duplicate (two plates) and two or three independent experiments performed at different times/days.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was not reported, but is assumed to have been done according to the publication(s) cited which describe the assay procedure.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methodology was not reported, but is assumed to have been done according to the publication(s) cited which describe the assay procedure.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	NA for this study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to this study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial 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	Low	× 1	3	The study indicates statistical analysis was performed using Student's t-test and results that were significant were reported in the text. SD or SE were not provided, preventing independent statistical analysis.
	Metric 23:	Data Interpretation	Medium	× 2	4	Scoring/evaluation criteria were not reported, but it appears that statistical analysis was the basis for positive/negative conclusions
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity was not included in the study design or discussed in the text and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.

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Study Citation:	M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures Journal of Applied Toxicology, 8(4,4), 243-248
Data Type:	Ames assay _CCL4 and chloroform
HERO ID:	194606

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Low	× 2	6	Positive control data were not reported. Results of statistical analysis and/or data to enable independent analysis (measure of variability) were not reported by strain or for all exposure concentrations.
Overall Quality Determination‡		Unacceptable⋆⋆		2.2	
Extracted		No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 28: **In vitro evaluation results of Wangenheim et al., 1988 for a study on mouse lymphoma mutagenicity assay**

Study Citation:	J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds Mutagenesis, 3(3,3), 193-205					
Data Type:	Mouse Lymphoma Mutagenicity Assay - CCl4					
HERO ID:	194626					
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	Supplied by E. Merck (FRG).
	Metric 3:	Test Substance Purity	Low	× 1	3	Study notes that chemicals were of the highest purity available; however, it does not report the actual purity of CCl4.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Six replicate solvent control agar plates were used for each chemical tested.
	Metric 5:	Positive Controls	High	× 2	2	4-Nitroquinoline-N-oxide was tested without metabolic activation and benzo[a]pyrene was tested with metabolic activation. Both gave expected results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay performed according to Clive et al., 1979 with some modifications that were briefly described. Some details were lacking (pre- and post-incubation temperatures, humidity, etc.)
	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	Methods were cited to another publication (Clive et al., 1979), and no additional details were provided regarding test substance preparation or storage. Lack of storage information is not likely to impact results as the test was performed for a short duration (4-hour exposure).
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were cited to another publication (Clive et al., 1979), and no additional details were provided regarding the application of CCl4.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported clearly as 0, 1.030E-3, 3.100E-3, and 4.130E-3 mol/L.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Treatment with the test substance was 4 hours and appropriate to the outcome of interest
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Justification for using 3 test concentrations and the concentrations chosen was briefly reported. 'Compounds were generally tested up to a concentration that reduced total growth to 10-20% of the solvent control except in those cases when low solubility precluded the achievement of toxic concentrations'. Four test concentrations are recommended for this assay type.

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Study Citation: J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds Mutagenesis, 3(3,3), 193-205
Data Type: Mouse Lymphoma Mutagenicity Assay - CCl4
HERO ID: 194626

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	CCl4 was only tested in the presence of metabolic activation. Aroclor 1254 pretreated male Sprague-Dawley rat liver homogenate (S9) was prepared in the lab or purchased from Litton Bionetics Inc., Kesington, MD, USA and was prepared according to Garner et al., 1972. Cofactors (not further specified) from Sigma Chemical Co. were added as described in Clive et al., 1979. Concentration or volume of S9 mix used was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Mouse lymphoma heterozygous L5178Y TK +/- 3.7.2.C cells were used and appropriate. Cells were obtained from Dr. Donald Clive of Burroughs Wellcome Co., Research Triangle Park, NC, USA.
	Metric 15:	Number per Group	High	× 1	1	3 replicates per treated culture were used and appropriate for a mammalian cell mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (manual and automatic colony counting) was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Referred to Clive et al., 1979. Assays where an irregular or absence of dose response occurred were retested. Approximately 20 chemicals were retested, but these were not specified.
	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 replicate or group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The results were subject to a statistical analysis. Tests for normal distribution of colony number (according to Shapiro and Wilk, 1965) were performed; ANOVA, and then pairwise two-tailed Student's t-test was performed to compare treated replicates versus the solvent control replicates. Table of results also provided for each concentration tested.

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Study Citation: J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds Mutagenesis, 3(3,3), 193-205
Data Type: Mouse Lymphoma Mutagenicity Assay - CCl4
HERO ID: 194626

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were briefly described and appropriate. Results were deemed positive if mutagenic activity was dose-related and statistically significant (1% or greater) when total growths were 10% or higher. Alternative interpretation using a 2-fold or greater increase in mutation frequencies with a 10% or higher growth was also considered.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Total growth (suspension growth x cloning efficacy) was evaluated based on methods described in Clive et al., 1979. Total growth was 20% at highest concentration tested., consistent with target survival for highest concentration (10-20%) reported by study authors
	Metric 25:	Reporting of Data	High	× 2	2	Table of results reported the total growth, mutation frequency, and mutation index with results of statistical analysis for all concentrations of CCl4 tested.
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 29: **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).
	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.

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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 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						
	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).

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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 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 30: **In vitro evaluation results of Araki et al., 2004**

Study Citation:	Araki, A; Kamigaitao, N; Sasaki, T; Matsushima, T (2004). Mutagenicity of carbon tetrachloride and chloroform in Salmonella typhimurium TA98, TA100, TA1535, and TA1537, and Escherichia coli WP2uvrA/pKM101 and WP2/pKM101, using a gas exposure method Environmental and Molecular Mutagenesis, 43(2), 128-133
Data Type:	
HERO ID:	194641

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and batch/lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative control was reported.
	Metric 5:	Positive Controls	Medium	× 2	4	The authors reported testing positive control substances by the pour plate method, but not the gas-phase exposure method.
	Metric 6:	Assay Procedures	High	× 1	1	Study authors described the methods and procedures.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Standards for test provided. The QC part of this test criteria may not be applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Authors reported the details of exposure administration.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors.
	Metric 13:	Metabolic Activation	High	× 1	1	Study authors reported exposures were conducted in the presence and absence of metabolic activation and the type and source, method of preparation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Authors provided descriptive information on the test model.
	Metric 15:	Number per Group	High	× 1	1	The authors provided details about the tester strains used in this study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The authors reported the outcome methodology for the study.

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Study Citation:	Araki, A; Kamigaitao, N; Sasaki, T; Matsushima, T (2004). Mutagenicity of carbon tetrachloride and chloroform in Salmonella typhimurium TA98, TA100, TA1535, and TA1537, and Escherichia coli WP2uvrA/pKM101 and WP2/pKM101, using a gas exposure method Environmental and Molecular Mutagenesis, 43(2), 128-133
Data Type:	
HERO ID:	194641

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling adequacy was reported for the outcome(s) of interest including more data values per dose group from different experiments.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Authors did not report any differences in study groups that was not related to chemical exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Authors reported statistical analysis of the data.
	Metric 23:	Data Interpretation	High	× 2	2	Authors followed the two-fold rule for mutagenicity in individual experiments.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The study authors reported cytotoxicity information.
	Metric 25:	Reporting of Data	High	× 2	2	Authors reported exposure-related findings as well as data from the negative controls.
Overall Quality Determination‡			~~High~~ ⟶ 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.

§ Evaluator's explanation for rating change: "A very well conducted study."

Table 31: **In vitro evaluation results of Hellmér et al., 1992 for a study on in vitro DNA repair test in E. coli**

Study Citation:	L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160					
Data Type:	CCl4 in vitro DNA repair test in E. coli					
HERO ID:	194717					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Carbon tetrachloride
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was not specifically reported, but it was noted that the chemicals tested were purchased from a commercial source. The product number and batch/lot number were also not reported; however, the material is not expected to vary in composition. The omitted details are unlikely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance were not reported. It was noted that all chemicals tested were of the highest purity obtainable.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Study authors report using a concurrent negative solvent control; however, the solvent used for carbon tetrachloride was not specified. This limitation is unlikely to have a substantial impact on results.
	Metric 5:	Positive Controls	High	× 2	2	Several compounds commonly used as positive controls were included in the study and produced positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described, but appear to be appropriate.
	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	The test substance preparation was reported; the solutions were made immediately before the experiment and did not need to be stored.
	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 highest concentration was reported as well as the dose where no surviving colonies were found and the dose where a statistically significant reduction of the number of colonies of each strain was seen .
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (1 day)

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Study Citation: L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
Data Type: CCl4 in vitro DNA repair test in E. coli
HERO ID: 194717

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups and dose/concentration spacing were justified by study authors (diluted in 7 half log steps or 2-fold dilution steps; but only 3 concentrations were specified (the highest concentration tested, the concentration where no surviving colonies were found, and the concentration where a statistically significant reduction of the number of colonies of each strain was seen) . Though not all exposure concentrations were reported, it is unlikely to have a substantial impact on results.
	Metric 13:	Metabolic Activation	High	× 1	1	Exposures were conducted in the presence and absence of a metabolic activation system. The source and method of preparation were reported.
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.
	Metric 15:	Number per Group	Low	× 1	3	The volume of bacterial mix was reported. One plate per concentration was tested.
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 for all three experiments.
	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 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						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical methods were described and appropriate for the dataset. It was noted that the confidence interval was determined according to the variance for each strain from a previous experiment; this data was not presented.
	Metric 23:	Data Interpretation	High	× 2	2	The scoring/evaluation criteria was reported (if the number of colonies was < 2 standard deviations of the mean for the strains, the test was considered significant).
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity endpoints were not defined.

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Study Citation:	L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
Data Type:	CCl4 in vitro DNA repair test in E. coli
HERO ID:	194717

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 25: Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study 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 32: **In vitro evaluation results of Onfelt 1987 for a study on aneuploidy**

Study Citation:	A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154
Data Type:	Aneuploidy-CCL4
HERO ID:	194719

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance (Carbon tetrachloride) was identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	Source for this compound is not clear.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported as p.a grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Pooled negative controls were used. The test substance was not diluted in a solvent, it is therefore assumed untreated negative controls were used although this was not explicitly stated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls were included but may not be necessary for this study type, the test substance gave a positive response
	Metric 6:	Assay Procedures	Medium	× 1	2	Limited, but sufficient methodological details were provided
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not necessary for this study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details on test substance preparation were provided. Details did not include mixing, addressing homogeneity or volatility, or storage.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were inferred from the text and assumed to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations (M) were reported however, the study indicated that the concentrations were inferred, or based on water solubility estimates given in the literature (1mg/2000mL). The stock solutions were assumed to be saturated based on this data.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (3 h for aneuploidy) was clearly reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Only two concentrations were tested, which is lower than the recommended three test groups.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for the study design
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	V79 Chinese hamster lung cells were an appropriate test model for the outcome of interest.

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Study Citation:	A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154
Data Type:	Aneuploidy-CCL4
HERO ID:	194719

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Medium	× 1	2	A single experiment (replicate) per exposure concentration was tested. Single treated cultures would considered acceptable if three concentrations were tested and the recommended number of cells were scored.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency across study groups was inferred from the text
	Metric 18:	Sampling Adequacy	Medium	× 2	4	For each concentration approximately 100-200 metaphase cells/slide were evaluated which is lower than the recommended 300 cells.
	Metric 19:	Blinding of Assessors	High	× 1	1	The test slides were reported to be coded.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in the test design or procedure were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The incidences of aneuploidy from treated samples were compared with controls using a Chi-square test.
	Metric 23:	Data Interpretation	High	× 2	2	A significant or positive score (increased incidence of aneuploidy) was based on statistical significance.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cell survival was reported as a % of control. Methodological details for determining survival are vague, cells were counted however, use of PI was not explicitly stated.
	Metric 25:	Reporting of Data	High	× 2	2	Data for all exposure groups was adequately presented.
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 $=\geq 1$ 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: "Suggested downgrade to medium for uncertainty about the exposure concentrations and only two exposure groups used in the study."

Table 33: **In vitro evaluation results of Onfelt 1987 for a study on aneuploidy**

Study Citation:	A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154
Data Type:	Aneuploidy-chloroform
HERO ID:	194719

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance (Chloroform) was identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	Source for this compound is not clear.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported as p.a grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Pooled negative controls were used. The test substance was not diluted in a solvent, it is therefore assumed untreated negative controls were used although this was not explicitly stated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls were included but may not be necessary for this study type; other test substances gave a positive response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Limited, but sufficient methodological details were provided
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not necessary for this study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details on test substance preparation were provided. Details did not include mixing, addressing homogeneity or volatility, or storage.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were inferred from the text and assumed to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations (M) were reported however, the study indicated that the concentrations were inferred, or based on water solubility estimates given in the literature (1ml/200mL). The stock solutions were assumed to be saturated based on this data.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (3 h for aneuploidy) was clearly reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Three exposure concentrations were tested, some discussion about the chosen concentrations was provided. The spacing appeared to be appropriate based on the study results.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for the study design
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	V79 Chinese hamster lung cells were an appropriate test model for the outcome of interest.

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Study Citation:	A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154
Data Type:	Aneuploidy-chloroform
HERO ID:	194719

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15: Number per Group	High	× 1	1	A single experiment (replicate) per exposure concentration was tested. Single treated cultures is considered acceptable if the recommended number of cells are scored.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome methodology was appropriate for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Consistency across study groups was inferred from the text
	Metric 18: Sampling Adequacy	Medium	× 2	4	For each concentration approximately 100-200 metaphase cells/slide were evaluated which is lower than the recommended 300 cells.
	Metric 19: Blinding of Assessors	High	× 1	1	The test slides were reported to be coded.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in the test design or procedure were identified.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	The incidences of aneuploidy from treated samples were compared with controls using a Chi-square test.
	Metric 23: Data Interpretation	High	× 2	2	A significant or positive score (increased incidence of aneuploidy) was based on statistical significance.
	Metric 24: Cytotoxicity Data	Medium	× 1	2	Cell survival was reported as a % of control. Methodological details for determining survival are vague, cells were counted however, use of PI was not explicitly stated.
	Metric 25: Reporting of Data	High	× 2	2	Data for all exposure groups was adequately presented.
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 $=\geq 1$ 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: "Suggested downgrade to medium for uncertainty about the exposure concentrations used in addition to other limited methodological details."

Table 34: **In vitro evaluation results of Oruambo et al., 1987 study on DNA binding – mouse liver chromatin**

Study Citation:	I. F. Oruambo, B. L. Van Duuren (1987). Distribution of carbon tetrachloride-metabolite(s) to DNase I-sensitive and -resistant chromatin Cancer Letters, 37(3,3), 311-316					
Data Type:	DNA binding – mouse liver chromatin					
HERO ID:	194721					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (radiolabeled [14C] CCl4)
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported New England Nuclear)
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported. However, the specific activity was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	The study design investigated binding regions of radiolabeled CCl4 in mouse chromatin; a negative control may not be required for this study.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control was not used; however, the study design investigated binding regions of radiolabeled CCl4 in mouse chromatin; a positive control may not be required for this study
	Metric 6:	Assay Procedures	Medium	× 1	2	Study authors described the methods and procedures; DNA isolation and purification methods were described in previously published studies.
	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	The test substance preparation was reported. Storage of the test substance was not reported; however, because it is a short-term study, this is appropriate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across the treated groups (2-hour and 4-hour groups)
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentration of the test substance was reported (2.5 umol [14C]CCl4)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the outcome of interest (2- and 4-hour incubations)
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Only one exposure concentration was tested for 2 exposure durations to measure DNA binding. This dose appeared to be sufficient for assessing the outcome of interest.
	Metric 13:	Metabolic Activation	Low	× 1	3	Exposures included microsomal protein; however, no descriptive details were reported
Domain 4: Test Model						

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Study Citation:	I. F. Oruambo, B. L. Van Duuren (1987). Distribution of carbon tetrachloride-metabolite(s) to DNase I-sensitive and -resistant chromatin Cancer Letters, 37(3,3), 311-316
Data Type:	DNA binding – mouse liver chromatin
HERO ID:	194721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The test model and descriptive information was reported (mouse liver chromatin)
	Metric 15:	Number per Group	High	× 1	1	Four replicates were included in the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest and was sensitive for that outcome
	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	Not Rated	NA	NA	Not applicable for this study design
	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	Information regarding the condition of mice the liver chromatin were derived from was not described, though the characteristics of isolated chromatin was.
	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. Results present the average and standard deviation of 4 experiments; independent analysis may be conducted.
	Metric 23:	Data Interpretation	High	× 2	2	Data was evaluated as the measure of metabolites bound to DNA in two regions of the chromatin structure.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Assay was conducted on chromatin DNA
	Metric 25:	Reporting of Data	Medium	× 2	4	Data was summarized as means and standard deviations of 4 experiments. Data for each experiment was not reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 35: **In vitro evaluation results of Díaz Gómez et al., 1981 study on DNA base interactions**

Study Citation:	M. I. Díaz Gómez, J. A. Castro (1981). Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases Research Communications in Chemical Pathology and Pharmacology, 32(1,1), 147-153
Data Type:	DNA Base Interactions with 14CCl4
HERO ID:	194767

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (radio-labeled [14C] CCl4)
	Metric 2:	Test Substance Source	High	× 1	1	14CCl4 was purchased from the Radiochemical Centre (England).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported. However, the specific activity was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A simultaneous control not containing the DNA base was included in the study design and processed in the same manner. The control value was subtracted from that of other treatment groups.
	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, pH, volume of saturated solution of each base used, etc.).
	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 of the test substance was described. [14C]CCl4 was added to an ampoule previously sealed under N2 with the DNA base saturated in 3 mL of absolute ethanol and 1 mg benzoyl peroxide for 16 hours at 80 deg C. Exposure duration was short (16 hours), therefore details on storage were not required.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It appears the same methods of exposure were used for each DNA base and control without the base.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentration of [14C]CCl4 was reported as 25.5E6 dpm/mL. Degradations per minute (dpm) can be converted to mCi and the quantity of [14C]CCl4 in mmol can be calculated based on the specific activity of 3.81 mCi/mmole.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was 16 hours, which appeared to be appropriate given that DNA binding was observed.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Justification for the concentration of [14C]CCl4 used was not reported., but appeared to be appropriate given that DNA binding was observed. One concentration tested in a DNA assay is acceptable.

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Study Citation:	M. I. Díaz Gómez, J. A. Castro (1981). Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases Research Communications in Chemical Pathology and Pharmacology, 32(1,1), 147-153
Data Type:	DNA Base Interactions with 14CCl4
HERO ID:	194767

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not required for this study type. Metabolic activation was not used.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The 4 DNA bases, guanine, adenine, cytosine, and thymine, were used and purchased from Sigma Chemical Co. The animal/cell line of origin for the DNA was not reported, but this is not expected to have substantially impacted results, given that they were obtained from a commercial source.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates per DNA base were not reported; however, appears to be a single assay per group. This is considered acceptable for a DNA binding assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology and results reported the interaction of trichloromethyl radical with the different DNA bases both by measuring the moles of 14C bound per mol of DNA base (table) and the reaction products (graphs).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	No inconsistencies were noted and it appears the outcomes were assessed consistently in each DNA base, although it was not specifically stated.
	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	Medium	× 2	4	Initial conditions were not reported for each study group, but this is not expected to have substantially impacted results.
	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	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria of radioactivity was described and results were reported in a table and graphs. Radioactivity was counted by adding water, NCS, PPO, in toluene to the fractions following evaporation. Column chromatographic analysis was performed on reaction products.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 25:	Reporting of Data	Medium	× 2	4	Results were provided for each DNA base (less the control value), although the results for the blank control without the DNA base were not reported.

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Study Citation:	M. I. Díaz Gómez, J. A. Castro (1981). Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases Research Communications in Chemical Pathology and Pharmacology, 32(1,1), 147-153
Data Type:	DNA Base Interactions with 14CCl4
HERO ID:	194767

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Overall Quality Determination[‡]		High		1.5	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 36: **In vitro evaluation results of Benigni et al., 1993 study on a somatic segregation assay**

Study Citation:	R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in Aspergillus nidulans Mutagenesis, 8(4,4), 301-305					
Data Type:	Somatic segregation assay for CCl4					
HERO ID:	194776					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported; however, may have been in a previously published study (Crebelli et al., 1992); it was indicated that CCl4 was re-tested in this study.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported; however, may have been reported in a previously published study (Crebelli et al., 1992); it was indicated that CCl4 was re-tested in this study.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an untreated solvent control (DMSO).
	Metric 5:	Positive Controls	Low	× 2	6	A positive control was tested (benomyl at 5 ug/mL) and induced increased numbers of whole chromosome segregants in abnormal colonies (not clear these data were analyzed statistically); in addition, survival for the positive control group was apparently reduced to 19%.
	Metric 6:	Assay Procedures	Low	× 1	3	Methods and procedures were not well-described; some aspects of the assay procedures were cited to other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	It can be inferred that the test substance was dissolved in solvent. Storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were described in a previously published paper (Crebelli et al., 1992).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Test concentrations were reported without ambiguity (Table II).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Assay methods were described in a previously published paper (Crebelli et al., 1992).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups concentration spacing were reported (5 plus controls) and justified by the study authors (based on a previously published study and re-tested in order to obtain more precise dose-response information).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for this study type.
Domain 4: Test Model						

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Study Citation:	R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in Aspergillus nidulans Mutagenesis, 8(4,4), 301-305
Data Type:	Somatic segregation assay for CCl4
HERO ID:	194776

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Test Model	Low	× 2	6	The test model was reported with limited descriptive information; test model characteristics may have been better described in a previously published study (Crebelli et al., 1992).
	Metric 15:	Number per Group	Not Rated	NA	NA	Assay methods were described in a previously published paper (Crebelli et al., 1992).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was described in a previously published study (Crebelli et al., 1992), though limited details appear appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment methodology was described in a previously published study (Crebelli et al., 1992), though limited details suggest that outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of colonies scored were reported (range 227 to 671).
	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	Information on the initial condition for each study group or replicate was 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	High	× 1	1	Statistical methods were reported used (chi-square test) and reported in Table II.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported; statistical analyses and the dose-relatedness of the response were factored into the determination of a positive response.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Toxicity was accounted for and measured as % surivival, though methods were not fully described.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome was presented quantitatively for the outcomes by exposure group.
Overall Quality Determination[‡]			Medium		1.9	
Extracted			Yes			

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Study Citation:	R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in Aspergillus nidulans Mutagenesis, 8(4,4), 301-305
Data Type:	Somatic segregation assay for CCl4
HERO ID:	194776

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 of Díaz Gómez et al., 1980 study on DNA binding**

Study Citation:	M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206
Data Type:	DNA binding of CCl4 metabolites
HERO ID:	194790

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 formula.
	Metric 2:	Test Substance Source	High	× 1	1	Source of the radiolabeled CCl4 (Radiochemical Centre, Amersham) was provided.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure as analyzed by glc analysis.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Controls are not needed for DNA binding studies.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Controls are not needed for DNA binding studies.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and/or cited in another publication 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	Medium	× 1	2	Preparation was described, but storage conditions were not indicated.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure appears consistent across groups/replicates.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Specific gravity of radiolabeled compound was provided. In an experiment where cold CCl4 concentration was added to the incubation, concentration was provided in mM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	30 minute exposure duration was appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	One experiment with radiolabeled compound; second experiment with cold CCl4 was added. This is adequate for the outcome of interest.
	Metric 13:	Metabolic Activation	High	× 1	1	Microsomal activation system was used.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Purified mouse liver DNA was not further described.
	Metric 15:	Number per Group	High	× 1	1	Triplicate simultaneous experiments were performed.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was reported (scintillation counting); this method is not very sensitive for the outcome of interest (compared to quantitative analysis for specific adducts).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.

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Study Citation:	M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206
Data Type:	DNA binding of CCl4 metabolites
HERO ID:	194790

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	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	Initial conditions were not reported across replicates or experiments.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	The footnote to Table 2 indicates that values are significantly higher than controls in Table 1; however no controls are presented in Table 1. No further details were provided.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported for exposed groups in each experiment; control results were noted in footnote to Table 3 but not reported. However, controls are not required for DNA binding studies.
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 38: **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						
	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.

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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 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						
	Metric 14:	Test Model	High	× 2	2	The cell lines used in the study were obtained from a commercial 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.

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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 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						
	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	

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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[††]
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 of Rocchi et al., 1973 for DNA binding**

Study Citation:	P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425
Data Type:	DNA binding for CCl4
HERO ID:	194878

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The chemical was identified by established nomenclature as carbon tetrachloride (radiolabeled).
	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	Test substance purity/grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Reactions without mouse or rat microsomes served as negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type; however, the test substance elicited positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	Procedures and test conditions were described in detail and appropriate for the study design. Other publications were cited for additional details.
	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	Appropriate volumes of undiluted test material were added to the reactions to obtain the desired specific activity/corresponding concentration desired in each reaction. Solutions were appropriately mixed. Test substance storage was not addressed (but not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were consistently administered across study groups. It appears that an additional exposure condition was used for activated mouse microsomes (10.9 umol in addition to 0.218 umol).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The concentrations tested were reported without ambiguity (e.g., 0.218 umol CCl4).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported (30 minutes) and considered appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	Although only one dose was used (for all conditions); however, the number of exposure groups was appropriate for the study design.
	Metric 13:	Metabolic Activation	High	× 1	1	Microsomes were extracted from rats and mice with and without metabolic activation. Details of activation and preparation were provided.
Domain 4: Test Model						

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Study Citation:	P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425
Data Type:	DNA binding for CCl4
HERO ID:	194878

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Test Model	Medium	× 2	4	The test model (rat and mouse microsomes) was appropriate for to evaluate the outcome of interest. The extraction of microsomes was cited to another publication.
	Metric 15:	Number per Group	Low	× 1	3	It was indicated that extraction of microsomes was performed on the liver of rats and mice in groups of 3. No additional information was provided.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was adequately described and appropriate for the outcome of interest. It was indicated that owing to stringent washing procedures, any binding detected represented true binding (the assessment was sensitive for the outcome of interest).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was evaluated 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. The outcome evaluated was not subjective.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in the test design and procedures 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	Not Rated	NA	NA	The data were not amenable to statistical analysis (only 1 test per condition). Data were presented as umol CCl4/mol P DNA.
	Metric 23:	Data Interpretation	Low	× 2	6	Details regarding data interpretation were limited. Without additional discussion, the data provided have less meaning.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design (no cells were used).
	Metric 25:	Reporting of Data	High	× 2	2	Data were provided for each exposure group (rat and liver microsomes, with and without activation, with and without pH enzymes).
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.6~~	
Extracted			Yes			

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Study Citation:	P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425
Data Type:	DNA binding for CCl4
HERO ID:	194878

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: "This study is downgraded to medium due to the absence of duplicate tests and an lack of details describing the requirements needed to consider the data positive for DNA binding given the reported background and test efficiency of 65

Table 40: **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- CCl4
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 Carbon tetrachloride ("CT") with the correct CASRN and molecular formula.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Aldrich and Fluka). 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). 99-99.9%
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
	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

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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- CCl4
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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 i n t he r eport. 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). F urthermore, t he u ncertainty r egarding t he 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.
	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						

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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- CCl4
HERO ID:	194881

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	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	
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 Roldán-Arjona and Pueyo 1993 for in vitro mutagenicity assay (Ara test) in S. typhimurium**

Study Citation:	T. Roldán-Arjona, C. Pueyo (1993). Mutagenic and lethal effects of halogenated methanes in the Ara test of Salmonella typhimurium: Quantitative relationship with chemical reactivity Mutagenesis, 8(2,2), 127-131
Data Type:	in vitro mutagenicity assay (Ara test) in S. typhimurium - CCl4
HERO ID:	194882

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (56-23-5); CCl4
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. 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 concurrent solvent (DMSO) control.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (2-aminoanthracene with S9 mixture).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described. The assay procedures were also described in a previously published study (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	Medium	× 1	2	Test substance preparation was described, though storage conditions were not.
	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
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (3 days).
	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 for the assays.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (S9 fraction from male liver induced with Aroclor-1254). Volume in the final culture was given. Method of preparation was cited in another publication.
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.

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Study Citation:	T. Roldán-Arjona, C. Pueyo (1993). Mutagenic and lethal effects of halogenated methanes in the Ara test of Salmonella typhimurium: Quantitative relationship with chemical reactivity Mutagenesis, 8(2,2), 127-131
Data Type:	in vitro mutagenicity assay (Ara test) in S. typhimurium - CCl4
HERO ID:	194882

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The number of cells were reported and appropriate.
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 for this study.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	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 methods were described and appropriate for the dataset.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cell survival was measured; however, the method of measurement was not explicitly reported.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data for the outcome was presented for the control and treatment groups; however, data for the positive control (2-AA) was not 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 42: **In vitro evaluation results of Galli et al., 1995 study on S. cerevisiae AGY3 DEL recombination**

Study Citation:	A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells Carcinogenesis, 16(3,3), 659-663					
Data Type:	S. cerevisiae AGY3 DEL Recombination – CCl4					
HERO ID:	194889					
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	CCl4 was purchased from Aldrich Chemical Company.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported, but because the test substance was obtained from a commercial source, this is not expected to have substantially affected results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative control was mentioned in Figures 1a and 1b as a 0.0 mg/mL concentration and in Table II. It was not reported if the control was untreated or solvent.
	Metric 5:	Positive Controls	High	× 2	2	Several chemicals were also tested in this assay and produced a positive response. Methyl methanesulfonate, ethyl methanesulfonate, and 4-nitroquinoline-1-oxide are all generally recognized as genotoxic and all produced positive responses, indicating the study was capable of detecting a positive response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described, but some details were lacking (humidity, washing methods, slide preparation, etc.).
	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	Study used the pre-incubation method under constant shaking. Preparation of the test substance was briefly described. Storage information was not required due to the short exposure duration (16-17 hours).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It was inferred that exposures were administered consistently across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Several concentrations were tested and noted on Figures 1a and 1b (appears to be 7 concentrations ranging from 0 to 10 mg/mL). Tabulated results were reported for 8 mg/mL.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Cultures were incubated for 16-17 hours, which appeared to be appropriate for assessing the outcome of interest, given the positive results in several test substances.

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Study Citation:	A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells Carcinogenesis, 16(3,3), 659-663
Data Type:	S. cerevisiae AGY3 DEL Recombination – CCl4
HERO ID:	194889

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Justification for the concentrations chosen was not reported. It is unclear how many concentrations were used due to the information being provided in a graph with multiple chemicals included in the same figure (appears to be 7 concentrations ranging from 0 to 10 mg/mL). Toxicity was apparent at the highest dose (% survival).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. No metabolic activation was used. Positive responses were observed.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Saccharomyces cerevisiae strain AGY3 was used for this assay and appears appropriate. Cell cultures were derived in-house and methods were described or referred to those of Gietz et al., 1992 and Rothstein, 1991.
	Metric 15:	Number per Group	Medium	× 1	2	Figure 1a and 1b indicated each experiment was repeated at least 3 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The number of recombinants were calculated per 10^4 cells for each colony (DEL events/10^4 survivors in Figure 1a and 1b). Methods for counting the number of recombinants was not reported. 'A minimum increase of 2-fold in a dose-dependent manner has been regarded as evidence for inducibility'.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	No inconsistencies were reported, although the outcome assessment methods were brief.
	Metric 18:	Sampling Adequacy	High	× 2	2	5 mL cultures containing 3E7 cells/mL were exposed to the test substances, and the number of recombinants were calculated per 10^4 survivors.
	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						

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Study Citation:	A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells Carcinogenesis, 16(3,3), 659-663
Data Type:	S. cerevisiae AGY3 DEL Recombination – CCl4
HERO ID:	194889

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Results were examined based on the fold over controls for the number of DEL recombinants and dose-response was examined. However, no statistical analysis was reported. The fold over control was only reported at 8 mg/mL in Table II. Because results without standard deviations both in the table and graphs, it would not be possible to conduct the statistical analysis/calculations independently.
	Metric 23:	Data Interpretation	High	× 2	2	'A minimum increase of 2-fold in a dose-dependent manner has been regarded as evidence for inducibility'.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	% survival was reported in a graph (Figure 1a), although numerical results were only provided at 8 mg/mL. Methods for counting the number of survivors was not reported.
	Metric 25:	Reporting of Data	Low	× 2	6	Figures 1a and 1b show the dose-response and % survival for CCl4, but it is difficult to distinguish exact values due to multiple chemical results being presented on the same graph. Only tabulated results were provided for 8 mg/mL. Quantitative results indicate positive results in the DEL assay for CCl4.
Overall Quality Determination‡			Unacceptable⋆⋆		1.7	
Extracted			Yes			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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: **In vitro evaluation results of Galli et al., 1998 study on chromosomal recombination**

Study Citation:	A. Galli, R. H. Schiestl (1998). Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells Mutation Research, 419(1-3,1-3), 53-68					
Data Type:	Chromosomal recombination for CCl4					
HERO ID:	194891					
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. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was identified. 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	The test substance purity/grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were run concurrently with each experiment. The negative control was presumably a solvent-only control (indicated in Table 4), but this was not explicitly specified for each independent experiment. The study authors noted that other chemical substances used in the recombination assay arrested in S phase were also considered negative controls (i.e., sodium azide, hydroxylamine hydrochloride, and ethanol).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not required by study type. However, a positive control (cyclophosphamide) was used in the assay that evaluated the role of activation. In addition, the other recombination assays used chemicals that were known carcinogens and/or had tested positive for recombination in previous assays. Test substances used in the assay produced positive responses, indicating that the test system is capable of detecting effects.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures (all recombination assays) were well-described (e.g., volumes, test conditions, incubation temperature) and appropriate for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	It was indicated that CCl4 was dissolved in DMSO (with no additional details). Storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for recombination assays was reported (i.e., 17 hours) and was appropriate for the study type.

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Study Citation: A. Galli, R. H. Schiestl (1998). Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells Mutation Research, 419(1-3,1-3), 53-68
Data Type: Chromosomal recombination for CCl4
HERO ID: 194891

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Exposure groups and spacing were appropriate for the outcome of interest (dose-response effects were observed). The justification for the selection of doses was not explicitly specified (presumably based on toxicity and/or previous studies).
	Metric 13:	Metabolic Activation	Low	× 1	3	The presence of metabolic activation (30% liver S9) was noted for one of the recombination assays. However, details of preparation were cited to another publication. The species of origin was also not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (Saccharomyces cerevisiae diploid strain RS112) was appropriate for the outcome of interest and described in detail. The strain appears to be laboratory-maintained (used in previous studies).
	Metric 15:	Number per Group	High	× 1	1	Reported results (all recombination assays) were from 2 to 3 independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was consistently assessed across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Confounding variables in test design and procedure were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	The study considered confounding factors (e.g., evaluated whether an interaction between HU and CCl4 affected results in S-arrested cells).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis was performed (Wilcox rank-sum test). Means and standard errors were reported.
	Metric 23:	Data Interpretation	High	× 2	2	Positive results were based on statistical significance or at least a significant two-fold induction with a positive dose-response.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity (cell viability) was reported however, the method used to assess cell viability was not specified.
	Metric 25:	Reporting of Data	High	× 2	2	Data for all exposure groups is adequately reported.
Overall Quality Determination[‡]			High		1.5	

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Study Citation:	A. Galli, R. H. Schiestl (1998). Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells Mutation Research, 419(1-3,1-3), 53-68
Data Type:	Chromosomal recombination for CCl4
HERO ID:	194891

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 44: **Animal toxicity evaluation results of Foureman et al., 1994 study on sex linked recessive lethal mutations in drosophila**

Study Citation:	P. Foureman, J. M. Mason, R. Valencia, S. Zimmering (1994). Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program Environmental and Molecular Mutagenesis, 23(3,3), 208-227
Data Type:	sex linked recessive lethal mutations in drosophila
HERO ID:	65173

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance is reported by name, CAS, and structure.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source and lot/batch is reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity is reported and adequate.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative control was reported; it is unclear if untreated or vehicle .
	Metric 5:	Positive Controls	Not Rated	NA	NA	Concurrent positive control is not applicable for this study type.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable for Drosophila.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of the test substance was reported, solutions were renewed at 24 and 48h.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported in Table 2 in ppm.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of exposure groups was selected based on solubility, palatability and toxicity (not further described) for a single dose (each route).
	Metric 12:	Exposure Route and Method	High	× 1	1	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 animals, drosophila, were reported and mating schematic was briefly described and appropriate for the study type.
	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	Number of animals in the study were reported and adequate for the outcome.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was briefly described, previously cited, and appeared adequate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type.

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Study Citation:	P. Foureman, J. M. Mason, R. Valencia, S. Zimmering (1994). Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program Environmental and Molecular Mutagenesis, 23(3,3), 208-227
Data Type:	sex linked recessive lethal mutations in drosophila
HERO ID:	65173

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study .
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Palatability was reportedly included in dose selection, but is not further described.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on health outcomes unrelated to exposure were not reported for each group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and were appropriate for the data.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all exposure 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 45: **In vitro evaluation results of Garry et al., 1990 for sister chromatid exchange**

Study Citation:	V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29
Data Type:	Sister chromatid exchange for CCl4
HERO ID:	194917

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (a manufacturer) was specified. 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	It was specified that the test substance was spectral grade.; observed effects are likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study reported that both untreated and solvent-only controls were used. It is not clear if dose '0' in Figure 1 pertains to the solvent-only control or untreated control. In addition, the solvent (Pluronic F127, a surfactant) is not routinely used (the difference in SCE response between untreated and solvent-only controls was not reported).
	Metric 5:	Positive Controls	Low	× 2	6	The study reported using a positive control (cyclophoshamide) in the presence of activation only (data not shown; presumably a positive response was observed). Although a positive control was not used in the absence of activation, other chemicals used in the study elicited a positive response without activation (indicating that the assay was at least capable of generating a positive response).
	Metric 6:	Assay Procedures	Medium	× 1	2	Most of the methods and procedures (e.g., test conditions, culture media and volumes, temperatures) used for the test were reported. Omissions (e.g., details regarding slide preparation) are not likely to substantially impact the study results.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (i.e., emulsified in surfactant). Stability of the test substance in the vehicle, which is not routinely used, was not reported. The study indicated that cells were exposed to the test substance in gas-tight glass vials to account for any volatility of the test substance.
	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 could be estimated from data presented graphically (Figure 1).

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Study Citation: V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29

Data Type: Sister chromatid exchange for CCl4

HERO ID: 194917

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure was specified (30 minutes), The duration of exposure was shorter than what is typically used for this study type (1 to 2 hours), the study indicated this duration was relevant to in vivo exposure.
	Metric 12:	Exposure Route and Method	High	× 1	1	The study reportedly used 4 doses and included testing up to a cytotoxic dose. The study indicated that doses were selected to delineate dose-response relationships. Based on the data presented in Figure 1, it appears that that there were 3 doses plus controls and that the highest dose was not analyzable (i.e., toxic) in the presence of activation.
	Metric 13:	Metabolic Activation	High	× 1	1	The study reported using metabolic activation (S9 rat liver homogenate); the source (a manufacturer) and concentration in final culture were also reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (human lymphocytes) was reported and is routinely used for the outcome of interest. The study indicated that blood donors were males aged 25 to 40, without evidence of chronic disease. The study identified several criteria that excluded subjects from the study.
	Metric 15:	Number per Group	High	× 1	1	The study reported using duplicate cultures.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology appeared appropriate for the outcome of interest. However, it was not entirely clear what stage of the cell cycle the cells were in; methods report that whole blood samples were treated then arrested with colcemid, but the legend for Figure 1 indicates that G0 lymphocytes were treated. The evaluation of SCEs after exposure of G0 cells (i.e., non-dividing cells) to the test substance may not have been sensitive for evaluating the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently among study groups.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Details regarding sampling of outcomes were not fully reported. The study indicated that "twenty cells per dose point were counted for SCEs." Typically, about 25 metaphases per culture are sampled (i.e., 50 per dose point with breakdown by culture). The study did not specify if only metaphases with 46 centromeres were analyzed.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not indicated.
Domain 6: Confounding / Variable Control						

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Study Citation:	V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29
Data Type:	Sister chromatid exchange for CCl4
HERO ID:	194917

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial differences with respect to the tissues exposed was not reported. The study only indicated that 5 cc of whole blood was used.
	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	Low	× 1	3	Statistical analysis was not described clearly (i.e., "analysis of variance procedures and regression analyses" were used).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study authors indicated that the criteria for a positive response was a dose-related increase in SCEs (expressed as SCEs/cell). Based on the information provided, it appears that this was determined by using a trend test .
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Toxicity was assessed, but methods were not well described.
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported graphically by exposure group for the mean number of SCEs per cell (without a measure of variation). The study authors did not present data in tabular form, and did not present results for numbers of SCEs (overall), or numbers of chromosomes/SCEs per chromosome.
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 46: **In vitro evaluation results of Garry et al., 1990 for chromosomal aberrations**

Study Citation:	V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194917

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (a manufacturer) was specified. 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	It was specified that the test substance was spectral grade.; observed effects are likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study reported that both untreated and solvent-only controls were used. It appears that only untreated controls were conducted concurrently with CCl4 treatments (Table 1 indicated "Solvent control" and "Control" for other compounds, and only "Control" for CCl4). In addition, the solvent (Pluronic F127, a surfactant) is not routinely used. The difference in CA response between untreated and solvent-only controls does not appear to be substantial, but it would instill more confidence if the effect of the surfactant was better described.
	Metric 5:	Positive Controls	Low	× 2	6	The study reported using a positive control (cyclophoshamide) in the presence of activation only (one data row shown in Table 1). Although a positive control was not used in the absence of activation, other chemicals used in the study elicited a positive response without activation (indicating that the assay was at least capable of generating a positive response).
	Metric 6:	Assay Procedures	Medium	× 1	2	Most of the methods and procedures (e.g., test conditions, culture media and volumes, temperatures) used for the test were reported. Omissions (e.g., details regarding slide preparation) are not likely to substantially impact the study results.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (i.e., emulsified in surfactant). Stability of the test substance in the vehicle, which is not routinely used, was not reported. The study indicated that cells were exposed to the test substance in gas-tight glass vials to account for any volatility of the test substance.
	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 (Table 1).

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Study Citation:	V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194917

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The duration of exposure was specified (30 minutes), The duration of exposure was shorter than what is typically used for this study type (3 to 6 hours), the study indicated this duration was chosen because it was relevant to in vivo exposure.
	Metric 12:	Exposure Route and Method	High	× 1	1	The study reportedly used 4 doses and included testing up to a cytotoxic dose. The study indicated that doses were selected to delineate dose-response relationships. Based on the data presented in Table 1, it appears that that there were 5 doses plus controls and that the highest dose was not analyzable (i.e., toxic) in the presence/absence of activation.
	Metric 13:	Metabolic Activation	High	× 1	1	The study reported using metabolic activation (S9 rat liver homogenate); the source (a manufacturer) and concentration in final culture were also reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (human lymphocytes) was reported and is routinely used for the outcome of interest. The study indicated that blood donors were males aged 25 to 40, without evidence of chronic disease. The study identified several criteria that excluded subjects from the study.
	Metric 15:	Number per Group	High	× 1	1	The study reported using duplicate cultures.
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	Outcomes were assessed consistently among study groups.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Details regarding sampling of outcomes were not fully reported. The study indicated that "one hundred cells per dose with 46 chromosomes were analyzed for aberrations." Typically, about 300 metaphases per dose are sampled (with breakdown by culture specified).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not indicated.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial differences with respect to the tissues exposed was not reported. The study only indicated that 5 cc of whole blood was used.
	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:	V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194917

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not described clearly (i.e., "analysis of variance procedures and regression analyses" were used).
	Metric 23:	Data Interpretation	Low	× 2	6	The study authors indicated that the criteria for a positive response was a dose-related increase in the mean number of aberrations per 100 cells (presumably based on pairwise comparisions from data in Table 1). However, it appears that data were interpreted in the context of untreated controls rather than solvent controls.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Toxicity was assessed, but methods were not well described.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported by exposure group for the mean number of aberrations per 100 cells. The study authors did not present data with respect to number of cells with aberrations, all types of aberrations (e.g., exchanges). number of aberrations including/excluding gaps.
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

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

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

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

Table 47: **In vitro evaluation results of Braun and Schoneich 1975 for Salmonella typhimurium mutagenicity in vitro spot-test**

Study Citation:	R. Braun, J. Schoneich (1975). The influence of ethanol and carbon tetrachloride on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with Salmonella typhimurium Mutation Research, 31(3,3), 191-194
Data Type:	S. typh. Mutagenicity, in vitro, spot-test – CCl4
HERO ID:	194934

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	Low	× 1	3	Source of CCl4 was not provided.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not reported.
	Metric 5:	Positive Controls	Unacceptable	× 2	8	A concurrent positive control group was not reported and test results were negative.
	Metric 6:	Assay Procedures	Low	× 1	3	The assay methods were very briefly described and lacked most details (test conditions, temperature, etc.).
	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	Test substance was dissolved in 0.9% saline; 0.1 mL spots were applied to center of petri dishes. It is unclear if volatility of the test substance was accounted for; lack of mitigation would substantially affect results. Storage information was not reported but this is unlikely to affect results as the test was performed for a short duration (spot test).
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Very limited exposure details were provided and it is unclear whether exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported as 10, 20, and 40 mg/mL.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Unacceptable	× 2	8	No information on exposure duration was reported.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Justification for the number of exposure groups and concentrations chosen was not reported. 3 concentrations were used. No information on cytotoxicity was provided so it is unclear whether the high concentration was sufficient as all concentrations yielded negative results.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was either not tested or not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Salmonella typhimurium his G46 and his TA1950 were used. These strains were provided by Professor B.N. Ames (Berkeley). Justification for selection of the strains was not provided.
	Metric 15:	Number per Group	Low	× 1	3	Number of replicates per group was not indicated.

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Study Citation:	R. Braun, J. Schoneich (1975). The influence of ethanol and carbon tetrachloride on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with Salmonella typhimurium Mutation Research, 31(3,3), 191-194
Data Type:	S. typh. Mutagenicity, in vitro, spot-test – CCl4
HERO ID:	194934

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Unacceptable	× 2	8	The outcome assessment methodology was not reported
	Metric 17: Consistency of Outcome Assessment	Low	× 1	3	The outcome assessment methodology and execution were not reported
	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 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	Not Rated	NA	NA	Statistical analysis was not conducted; however, it is not necessarily required for a bacterial mutation test.
	Metric 23: Data Interpretation	Low	× 2	6	Evaluation criteria were not reported.
	Metric 24: Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity was not defined or described and it is unclear if cytotoxicity was accounted for in this study.
	Metric 25: Reporting of Data	Low	× 2	6	Negative results were reported qualitatively, but no data or additional details were provided. Results were not provided for each group.
Overall Quality Determination‡		Unacceptable**		3.4	
Extracted		No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 of Brennan and Schiestl 1998 for intrachromasomal recombination in yeast**

Study Citation:	R. J. Brennan, R. H. Schiestl (1998). Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in Saccharomyces cerevisiae Mutation Research, 397(2,2), 271-278					
Data Type:	Intrachromasomal recombination in yeast					
HERO ID:	194935					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substances (carbon tetrachloride and chloroform) were identified by name and the correct CASRNs.
	Metric 2:	Test Substance Source	High	× 1	1	Test substances were obtained from a commercial source (Aldrich)
	Metric 3:	Test Substance Purity	Medium	× 1	2	It was unclear whether "99%+ pure" referred to CCl4 or all test substances listed. However, because chloroform was also obtained from a commercial source, this is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls reported; while not explicitly described, no vehicle was used (chemical mixed in medium) so an untreated control is appropriate.
	Metric 5:	Positive Controls	High	× 2	2	4-nitroquinoline N-oxide used as positive control
	Metric 6:	Assay Procedures	Medium	× 1	2	Test conditions reported in detail including cell concentration, equipment, solution concentrations, incubation period and temperature, and plating. Medium preparation was cited to another publication.
	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	Preparation described (mixed in SC-leu medium) but storage was not reported. This is appropriate given the short duration of study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were consistent (cell concentrations, equipment, volumes, etc.)
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported as mg/mL in table 1.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Cells incubated for 17 hr. This duration was sufficient to induce recombinations with the positive control.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Four doses for CCl4 and 5 doses for CHCl3 were used. The high dose for both test substances was very toxic (about 1% survival or less) and even the lowest doses reduced survival by ~50%. This is considered to have substantially impacted results. However, it is still considered acceptable because 1) the positive control gave a positive response even with survival at 8-18%, and because both test substance showed a dose-dependent response despite the low survival.

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Study Citation: R. J. Brennan, R. H. Schiestl (1998). Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in Saccharomyces cerevisiae Mutation Research, 397(2,2), 271-278
Data Type: Intrachromasomal recombination in yeast
HERO ID: 194935

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Positive responses observed without metabolic activation
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was Saccharomyces cerevisiae and strain genotype was described in detail. Standard species for recombination assays.
	Metric 15:	Number per Group	Low	× 1	3	Table 1 reports results from "at least two" replicates. Two replicates is considered somewhat lacking, and the vague "at least two" phrase adds uncertainty to this metric.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment cited to another publication with no additional details
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment cited to another publication with no additional details
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Outcome assessment cited to another publication with no additional details
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No information on blinding was reported. Outcome assessment cited to another publication with no additional details
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms 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	Low	× 1	3	Statistical analysis was not conducted. Means and standard deviations were reported, but sample sizes ("at least two" replicates) were unclear, so it is not possible to conduct independent statistical analysis. However, this is still considered acceptable, as statistical analysis is not necessarily required for recombination assay (2-fold increase considered positive).
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria reported (2-fold increase considered positive) and cited to other publications.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Survival evaluation methods were cited to another publication. Cell survival was reported for all doses and control
	Metric 25:	Reporting of Data	High	× 2	2	Survival and DEL recombination frequencies reported for all doses and assay conditions; means and SDs reported; results for negative and positive controls reported.
Overall Quality Determination[‡]			High		1.5	

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Study Citation:	R. J. Brennan, R. H. Schiestl (1998). Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in Saccharomyces cerevisiae Mutation Research, 397(2,2), 271-278
Data Type:	Intrachromasomal recombination in yeast
HERO ID:	194935

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 49: **In vitro evaluation results of Khudoley et al., 1987 for bacterial reverse mutation study**

Study Citation:	V. V. Khudoley, I. Mizgireuv, G. B. Pliss (1987). The study of mutagenic activity of carcinogens and other chemical agents with Salmonella typhimurium assays: Testing of 126 compounds Archiv für Geschwulstforschung, 57(6,6), 453-462					
Data Type:	Bacterial reverse mutation for CCl4					
HERO ID:	194949					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride with the correct CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of CCl4 was not reported. A subset of the 126 test substances were reported to have been synthesized at the home institution of the authors, so it can be assumed that the CCl4 was obtained from an unidentified commercial source.
	Metric 3:	Test Substance Purity	Low	× 1	3	It was reported that the "majority" of the 126 test substances were "chemically pure". The purity of CCl4 was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were included concurrently in study design.
	Metric 5:	Positive Controls	Low	× 2	6	Appropriate concurrent positive control test substances were included for each test condition with and without S9 activation. Positive control data were not reported.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay methods and procedures were cited to other publications.
	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	Assay methods were cited to other publications.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Assay methods were cited to other publications.
	Metric 10:	Reporting of Doses/Concentrations	Not Rated	NA	NA	Assay methods were cited to other publications.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The assay procedures were described as "routine protocol" and cited in other references.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	The number of exposure groups and dose spacing were not reported. The assay procedures were described as "routine protocol" and cited in other references.
	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.
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.

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Study Citation:	V. V. Khudoley, I. Mizgireuv, G. B. Pliss (1987). The study of mutagenic activity of carcinogens and other chemical agents with Salmonella typhimurium assays: Testing of 126 compounds Archiv für Geschwulstforschung, 57(6,6), 453-462
Data Type:	Bacterial reverse mutation for CCl4
HERO ID:	194949

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Not Rated	NA	NA	The number of plates per treatment group was not reported. The assay procedures were described as "routine protocol" and cited in other references.
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.
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	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	Medium	× 1	2	The data were statistically analyzed, but the statistical test was not reported. A positive result was defined as a dose-dependent response at least 2x background mutation rates, which is appropriate for this study design.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	No cytotoxicity assay was included for the bacterial mutagenicity assay; however, this is unlikely to have a substantial impact on the study results.
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination[‡]			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 50: **In vitro evaluation results of Levy and Brabec 1984 for mitochondrial and nuclear DNA binding**

Study Citation:	G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234
Data Type:	Mitochondrial and nuclear DNA binding for CCl4
HERO ID:	194952

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 14C-labelled carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Amersham). 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 radiochemical purity of the test substance was reported (>99%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	The use of a negative control group is strictly required; measurement of the radiolabeled test compound is the outcome.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not required by study type; however, treatment-related results were observed (indicating that the assay was effective).
	Metric 6:	Assay Procedures	Low	× 1	3	Assay methods and procedures were partially described. Mitochondria preparation was cited to another publication (Brabec et al., 1975). Details of mitochondrial DNA preparation were omit-ted. Information about the assay using calf thymus nuclear DNA were not provided.
	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	Details regarding test substance preparation were limited. Storage was not reported (but not expected to impact study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It is inferred that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The dose was reported without ambiguity (30 mM CCl4 containing 25 uCi 14C-labelled CCl4).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (30 minutes) and appeared adequate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	One concentration was used; however, this concentration was sufficient to detect DNA binding.
	Metric 13:	Metabolic Activation	Low	× 1	3	The presence of a metabolic activation system was reported (post-mitochondrial fraction), but was not validated. It is not clear if the PMS is an adequate activation condition for calf thymus DNA (especially in the absence of a positive response).
Domain 4: Test Model						

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Study Citation:	G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234
Data Type:	Mitochondrial and nuclear DNA binding for CCl4
HERO ID:	194952

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Test Model	Low	× 2	6	The test models were reported with limited (mitochondrial suspension) to no (calf thymus DNA) additional information.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that results for mitochondrial DNA were based on 4 experiments and results using calf thymus DNA were based on 3 experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Information on initial conditions for each study 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 analysis was not conducted. Data were provided as means +/- standard deviations for a specified number of experiments (n = 3 or 4).
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not explicitly specified (other than the detection of radiolabel in isolated DNA).
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study type (no cells were used).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination[‡]			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 51: **In vitro evaluation results of Castro et al., 1989 for DNA and nuclear protein binding**

Study Citation:	G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294
Data Type:	DNA and nuclear protein binding
HERO ID:	194983

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as CCL4, CASRN provided. Radiolabeled CCL4 also used.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial sources were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Unlabeled CCL4 reported to be "low sulfur quality", - labeled CCL4 purity 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Not applicable for the study design (DNA binding/adduct assays)
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for the study design
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were cited to another publication but with some details briefly described.
	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	Details of exposure were described. Storage of radiolabeled CCL4 was reported and stability was tested/confirmed
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were consistent for each test condition and performed under an N2 atmosphere.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentration was reported (0.2mM radiolabeled CCL4)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The reaction duration (1hr) was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	Only a single dose tested, however, this was appropriate for the study design and the outcome of interest.
	Metric 13:	Metabolic Activation	High	× 1	1	Experiments were performed in the presence and absence of an NADPH generating system.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Nuclear DNA and protein preparations from livers of three species (mouse, rat, and hamster)
	Metric 15:	Number per Group	High	× 1	1	Experiments in each species were performed in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Assessment methods were cited to another publication, but details were briefly described and the methods appeared appropriate.

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Study Citation:	G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294
Data Type:	DNA and nuclear protein binding
HERO ID:	194983

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment appeared to be consistent across species.
	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	Low	× 2	6	Confounding variables in test design and procedures were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Purity of the nuclear preparations were tested and confirmed
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis to evaluate differences between species and results in the presence and absence of NADPH was performed and appropriate (student T-test). Data were presented as Means with SD
	Metric 23:	Data Interpretation	High	× 2	2	Data interpretation was appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Not applicable (no cells)
	Metric 25:	Reporting of Data	High	× 2	2	All experimental data was 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 52: **In vitro evaluation results of Coutino 1979 for chromosomal abnormalities**

Study Citation:	R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136					
Data Type:	CA-CCL4					
HERO ID:	195013					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name as CCL4, CASRN not provided.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source not reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent non-solvent (buffer) controls were used as comparators. DMSO was included in the test, but as one of the test substances not as a control. The text suggests compounds were added undiluted or in PBS, it is therefore assumed no solvents were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The study did not specify use of a positive control, however a positive responses were indicated/ reported in the text for several compounds..
	Metric 6:	Assay Procedures	High	× 1	1	The Assay methods were sufficiently described and were appropriate for the outcome of interest.
	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	Low	× 1	3	Limited details of test substance preparation (added with PBS or alone) and no details of test substance storage were reported. Test substance was noted as being volatile, however, test procedures do not indicate any measures were taken to account for volatility during exposure.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistency across groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The study indicates that logarithmic or geometric progressions of concentration were evaluated, however only the dose (5ul/mL) producing the highest frequency of anomalies are reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (24hrs) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study indicates that logarithmic or geometric progressions of concentration were evaluated, however the specific number of doses tested was not reported. No further justification of doses were provided.
	Metric 13:	Metabolic Activation	Low	× 1	3	Metabolic activation was not included; metabolites were not directly tested, however a positive response was observed.
Domain 4: Test Model						

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Study Citation: R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136
Data Type: CA-CCL4
HERO ID: 195013

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Test Model	High	× 2	2	The test model (CHO cells) is appropriate for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	The study indicates there were two dishes/dose however, the study does not indicate these were duplicates, but rather the number of dishes required to obtain the number of cells needed for the outcome assessment.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was described in limited detail (e.g., cells were analyzed). No additional information was provided.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Details of outcome assessment were limited, although it is assumed the the controls and samples were consistently evaluated.
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of cells analyzed per group was appropriate (500 anaphases)
	Metric 19:	Blinding of Assessors	Low	× 1	3	Use of blinding or coded cells were not reported and should be included in this type of study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in the test design or procedure were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables not related to the outcome of exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not included (single dose tested)
	Metric 23:	Data Interpretation	Low	× 2	6	Details of scoring or evaluation criteria were not described. The text indicates there is a "great increase" without indicating what the threshold is for an increase is.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity was measured indirectly by means of the mitotic index, but additional details of the procedure/assessment were not reported.
	Metric 25:	Reporting of Data	Low	× 2	6	The text indicated that no compounds had cytotoxic effects at the concentrations producing anomalies, but the data were not provided. Experimental Data were reported as ratios to control. Percentages or incidence data for the exposure group were not reported.
Overall Quality Determination[‡]			Medium		2.0	
Extracted			Yes			

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Study Citation:	R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136
Data Type:	CA-CCL4
HERO ID:	195013

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 53: **In vitro evaluation results of Gualandi 1984 for somatic segregation**

Study Citation:	G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type:	Somatic segregation for CCl4 and chloroform
HERO ID:	195130

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were clearly identified as carbon tetrachloride (CCl4) and chloroform (CHCl3).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substances was reported (Merck).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substances were reportedly analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group (presumably untreated).
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not tested; however, treatment-related positive responses were observed demonstrating that the test is capable of detecting a positive response.
	Metric 6:	Assay Procedures	Low	× 1	3	Methods and procedures were reported in limited detail. Media preparation was cited to a previous publication (Gualandi and Morpurgo, 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	Low	× 1	3	Storage was not reported (but was not expected to impact the study results). However, there were substantial deficiencies regarding test substance preparation. It appears that test chemicals were added to molten agar. The study indicates that the test substances were not soluble in aqueous media.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Exposures appeared to be administered consistently across study groups; however, there was not enough information provided to determine if consistent volumes were used. The study also indicated that CCl4 decays in the plate (i.e., no control for evaporation).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration used in this assay was reported without ambiguity (0.5% v/v).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration of the plate-mediated somatic segregation assay was not clearly reported (2 to 3 days).
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Based on information presented in the results, it appears that the study authors intended to use more than one concentration; however, since the test substances were not soluble in aqueous media, only a narrow range of concentrations could be tested. Only one concentration was tested, and toxicity at this dose was >70% for both test substances.

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Study Citation: G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type: Somatic segregation for CCl4 and chloroform
HERO ID: 195130

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type (however, fungi possess a cytochrome P-450-dependent monoxygenase system).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited (mainly genotypic) information. Some information pertaining to the diploid strain was cited to another publication (Gualandi and Morpurgo 1983).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that there were 7 replicates for CCl4 and 4 replicates for CHCl3.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was reported in limited detail. It was indicated that the assay was sensitive for the outcome of interest. Cross-overs and deletions were indistinguishable for the test.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.
	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	Information on the initial conditions for each study group or replicate was 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	High	× 1	1	No statistical analysis was conducted; however, means SEMs, and numbers of replicates were provided for independent analyses.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not described. Based on information presented in the results and discussion, fold-changes over controls were considered as a factor for determining a positive response (e.g., a two- to three-fold increase was considered weakly positive).
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study authors provided data as % surivival; however, cytotoxicity methods were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome was presented quantitatively for the outcome by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	

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Study Citation:	G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type:	Somatic segregation for CCl4 and chloroform
HERO ID:	195130

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

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 54: **In vitro evaluation results of Gualandi 1984 for gene mutations**

Study Citation:	G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type:	Gene mutation CCl4 and chloroform
HERO ID:	195130

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were clearly identified as carbon tetrachloride (CCl4) and chloroform (CHCl3).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substances was reported (Merck).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substances were reportedly analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group (presumably untreated).
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not tested; however, (weakly) positive responses were observed demonstrating that the test is capable of detecting a positive response.
	Metric 6:	Assay Procedures	Low	× 1	3	Methods and procedures were reported in limited detail. Media preparation was described in a previous study (Gualandi and Morpurgo, 1983) and the growth-mediated assay cited Bignami et al., (1981).
	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 was not expected to impact the study results). However, there were substantial deficiencies regarding test substance preparation. It appears that test chemicals were added to molten agar. The study indicates that the test substances were not soluble in aqueous media.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposures appeared to be administered consistently across study groups; however, there was not enough information provided to determine if consistent volumes were used. The study also indicated that CCl4 decays in the plate (i.e., no control for evaporation).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration used in this assay was reported without ambiguity (0.5% v/v).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Exposure duration were not clearly reported (i.e., 4 to 5 days for the growth-mediated assay and/or until sporulation).
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Based on information presented in the results, it appears that the study authors intended to use more than one concentration; however, since the test substances were not soluble in aqueous media, only a narrow range of concentrations could be tested. Only one concentration was tested, and toxicity at this dose was approximately 70% for both test substances.

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Study Citation: G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type: Gene mutation CCl4 and chloroform
HERO ID: 195130

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type (however, fungi possess a cytochrome P-450-dependent monoxygenase system).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited (mainly genotypic) information. Additional information pertaining to the haploid strain was cited to another publication (Lilly 1965).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that there were 2 to 3 replicates for CCl4 and CHCl3.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was reported in limited detail. It was indicated that the assay was not very sensitive for the outcome of interest (especially the plate incorporation assay).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.
	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	Information on the initial conditions for each study group or replicate was 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	High	× 1	1	No statistical analysis was conducted; although means and SEMs were provided, independent analyses could not be performed without information on the specific number of replicates (2 or 3). However, for mutation assays, fold-changes rather than statistical analyses are often used to analyze the study results.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not described. Based on information presented in the results and discussion, fold-changes over controls were considered as a factor for determining a positive response (e.g., a two- to three-fold increase was considered weakly positive).
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study authors provided data as % surivival; however, cytotoxicity methods were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome was presented quantitatively for the outcomes by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	

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Study Citation:	G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in Aspergillus nidulans Mutation Research, 136(2,2), 109-114
Data Type:	Gene mutation CCl4 and chloroform
HERO ID:	195130

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

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 55: **In vitro evaluation results of Whittaker et al., 1989 for chromosome loss in yeast**

Study Citation:	S. G. Whittaker, F. K. Zimmermann, B. Dicus, W. W. Piegorsch, S. Fogel, M. A. Resnick (1989). Detection of induced mitotic chromosome loss in Saccharomyces cerevisiae--an interlaboratory study Mutation Research, 224(1,1), 31-76
Data Type:	Chromosome loss - Yeast
HERO ID:	198010

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as carbon tetrachloride; the CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial supplier (Radian Corp) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated and solvent (ethanol) controls were included.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls (methyl benzimidazol-2-yl-carbamate) or propionitrile) were used and responded as expected.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail and were appropriate for the outcome of interest.
	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	Test substance preparations (dilutions from stock) were adequately described and were performed just prior to addition to the cultures. Storage (of stock solutions) was not described. Reactions were performed in sealed containers to account for potential volatilization.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure were reported and methods were consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Test concentrations were clearly reported in mg/mL.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration for each type of test was clearly reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure groups and spacing were determined based on a pretest to identify inhibiting concentrations. Up to 10 doses were tested (minimum 4) in each experiment
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for test system (yeast)
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (diploid yeast strain D6.1M) was reported and appropriate
	Metric 15:	Number per Group	High	× 1	1	Experiments were performed in triplicate, and some were performed by two independent laboratories.
Domain 5: Outcome Assessment						

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Study Citation:	S. G. Whittaker, F. K. Zimmermann, B. Dicus, W. W. Piegorsch, S. Fogel, M. A. Resnick (1989). Detection of induced mitotic chromosome loss in Saccharomyces cerevisiae--an interlaboratory study Mutation Research, 224(1,1), 31-76
Data Type:	Chromosome loss - Yeast
HERO ID:	198010

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was clearly detailed and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent between treatment groups and controls.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to study type (colonies counted)
	Metric 19:	Blinding of Assessors	High	× 1	1	The chemicals tested were coded prior to the start of the experiments.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions (e.g., batch/lot number) per group were 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	The methods of statistical analysis were reported. Justification for the methods were provided.
	Metric 23:	Data Interpretation	High	× 2	2	The study authors clearly described evaluation criteria.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Pre-tests were done to determine the effect of concentrations on cell titers. Cell viability was calculated from the number of colonies on synthetic complete media. Viable titers were reported concurrently with each experiment.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data for all tests and exposure groups were reported without a measure of variability across replicates
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 56: **In vitro evaluation results of Barber et al., 1981 for bacterial reverse mutation**

Study Citation:	E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48
Data Type:	Bacterial reverse mutation for CCl4
HERO ID:	200219

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 carbon tetrachloride. A structure was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (Eastman Organic Chemicals). A batch/lot number was not reported, but the chemical substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of CCl4 as per GLC was 98.5%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls consisted of plates in a closed system with no added test or positive control chemical. With the exception of not adding chemical to the system, untreated controls were treated the same as treatment groups. Negative controls were used for each strain, with and without metabolic activation.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used. It is noted that positive control substances were not volatile, and were (therefore) not subjected to a closed test system. 2-Aminoanthracene was the positive control with activation (all strains). Without activation, ICR-191 was used for S. typhimurium TA 98, methyl-N-nitro-N'-nitroguanidine was used for strains TA 100 and TA 1535, 9-aminoacridine was used for TA 1537, and picrolonic acid was used for TA 1538. Positive controls yielded positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	In this study, a modified plate-incorporation test was conducted using a chemically inert, closed-system protocol. Assay methods were described in detail, including the system used and how the addition of CCl4 was handled.
	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	Owing to the volatility of the test substance, doses were confirmed. Plates containing only distilled water were included in the closed system for GLC analysis of aqueous CCl4 concentrations at the end of the 48-hour incubation period Samples of the vapor were also taken from the closed system containers at the end of the period and analyzed by GLC.
	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. Measured CCl4 concentrations were 0, 4.7, 5.7, 10.2, 12.3, and/or 18.4 µmoles/plate.

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Study Citation:	E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48
Data Type:	Bacterial reverse mutation for CCl4
HERO ID:	200219

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate. Plates were exposed for 48 hours at 37C. The study generated conditions that permitted the tester strains to be exposed to CCl4 as a vapor for the entirety of the 48-hour exposure period (without loss due to volatility).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups was adequate for the study type (at least 4 exposure concentrations plus controls); however, the range of doses was lacking as it covered less than a full order of magnitude. Results were negative at all doses; although the text indicates that the test substance was tested at concentrations sufficient to produce observable toxicity to the tester strains, toxicity is not indicated for CCl4 in Table 4.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Aroclor-induced rat liver S9 was used. The source was reported (a manufacturer). Details regarding composition were not provided.
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.
	Metric 15:	Number per Group	High	× 1	1	Table 6 suggests that 5 replicates were used per group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest. The number of revertant colonies/plate was counted after 48 hours incubation. Revertant colonies were counted using a colony counter.
	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 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 identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Increased revertants/plate compared to controls was evaluated using statistical analysis (Student's t-test). Statistics were used to determine the minimum vapor concentration that significantly increased the number of revertant colonies.

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Study Citation:	E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48
Data Type:	Bacterial reverse mutation for CCl4
HERO ID:	200219

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) were reported. The criteria for a positive result was increased revertants/plate compared to controls (analyzed statistically).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity was described as absence of a background lawn. Further details were not provided.
	Metric 25:	Reporting of Data	Medium	× 2	4	Average spontaneous reversion rates from negative controls were reported (and were reportedly in agreement with those found by an interlaboratory survey by de Serres and Shelby [1979] and those presented by Ames [1975]). Raw data (i.e., individual plate counts) were not provided. Negative data were reported qualitatively (i.e., for S. typhmurium strains TA 1537 and TA 1538). Standard deviations for mean numbers of revertants/plate (except positive and negative controls) were not reported. No historical control data was provided.
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 57: **In vitro evaluation results of Crebelli et al., 1988 for Aspergillus mitotic segregation**

Study Citation:	R. Crebelli, R. Benigni, J. Franekic, G. Conti, L. Conti, A. Carere (1988). Induction of chromosome malsegregation by halogenated organic solvents in Aspergillus nidulans: Unspecific or specific mechanism? Mutation Research, 201(2,2), 401-411
Data Type:	Aspergillus mitotic segregation_ CCl4
HERO ID:	200282

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) and CASRN (56-23-5).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (purchased from Carlo Erba, Milan). Although a batch/lot number was not provided, the substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (>99.5%); any observed effects are highly likely caused by the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported the use of negative controls; all conditions (except for addition of the test substance) appeared to be equal.
	Metric 5:	Positive Controls	Medium	× 2	4	A positive control (benomyl) was reported. There were uncertainties associated with the use of this control group. Data for the positive control were shown in Table 2 only (data for CCl4 in Table 1); Table 2 references to historical control values for the positive control whereas the methods indicate the chemical was used in the study (not entirely clear if the control was concurrent, and no statistics were applied to these data). These uncertainties are not expected to substantially affect the study results.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods and procedures were partially described and/or attributed to other cited publications (e.g., classification of yellow segregants). The procedures appear to be applicable to the study type, and omissions (e.g., cell density) are unlikely to substantially impact the study results.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	The metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Minimal details regarding test substance storage and/or preparation were reported. The study indicates that conidia were treated with the test substance in sealed capped tubes. The lack of additional details is not expected to substantially impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.

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Study Citation: R. Crebelli, R. Benigni, J. Franekic, G. Conti, L. Conti, A. Carere (1988). Induction of chromosome malsegregation by halogenated organic solvents in Aspergillus nidulans: Unspecific or specific mechanism? Mutation Research, 201(2,2), 401-411
Data Type: Aspergillus mitotic segregation_ CCl4
HERO ID: 200282

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration appeared to be appropriate for the study type. The study indicated that this protocol is routinely used. Pre-germinating conidia were treated the test substance until the emergence of the germ tube (approximately 3 hours).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (5 + control) and concentration spacing were justified by the study authors and appeared to be be adequate to address the purpose of the study. The study indicated that a wide range of concentrations was applied to determine the lowest and highest effective doses as well as the lowest concentration arresting conidial germination or inducing a lethal hit per cell.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The metric is not applicable to this study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The strain was generated (and was presumably maintained) by the laboratory that conducted the study. Limited descriptive information about the strain (A. nidulans diploid strain P1) was provided (i.e., genetic information). The study indicates that the test model organism is a common choice for the detection of chemically induced chromosome missegregation.
	Metric 15:	Number per Group	Medium	× 1	2	The study does not make reference to replicates; there may have been only one per exposure group. However, this limitation is unlikely to substantially impact the study results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment addressed the intended outcome of interest (i.e., the frequency of mitotic segregants).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment protocol was applied consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Uncertainties were identified with respect to the outcome of interest. A large number of colonies were scored. However, the number of colonies scored ranged from 2371 in controls to only 182 in the highest exposure group (presumably due to decreased germination at higher concentrations).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The metric is not applicable to this study type.
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:	R. Crebelli, R. Benigni, J. Franekic, G. Conti, L. Conti, A. Carere (1988). Induction of chromosome malsegregation by halogenated organic solvents in Aspergillus nidulans: Unspecific or specific mechanism? Mutation Research, 201(2,2), 401-411
Data Type:	Aspergillus mitotic segregation_ CCl4
HERO ID:	200282

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were applied to the data, and appeared to be appropriate for the study type. Statistical significance was clearly reported in the data table (p< 0.05 or P<0.001 based on chi-square test). Raw data were provided, enabling independent statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	The study indicated that "positive" mitotic segregants were detected as homo- or hemizygous yellow sectors or patches in heterozygous pale green colonies. Segregants were further classified as mitotic crossovers or non-disjunctional diploids or haploids. These evaluation criteria appear to be consistent with routine methods for this study type.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study identified the lowest exposure concentration that arrested conidial germination for other test substance, but this was not seen at the doses tested for CCl4. The study authors suggested that increased missegregation was induced at concentrations that affected cell division, but did not block division (i.e., at doses up until arrest was observed).
	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 58: **In vitro evaluation results of Garrett and Lewtas 1983 for inhibition of DNA and protein synthesis**

Study Citation:	N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465
Data Type:	Inhibition of DNA and protein synthesis for CCl4
HERO ID:	626038

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 carbon tetrachloride.
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance was commercially sourced. Although the name of the manufacturer was not reported, this omission is not likely to substantially impact the study results.
	Metric 3:	Test Substance Purity	High	× 1	1	The specific purity of the test substance was not reported, but it was noted that every chemical tested was "reagent grade and the highest purity commercially available."
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative solvent controls were included. It is noted that water insoluble compounds were dissolved "with small amounts of acetone, ethanol, or DMSO;" it was not specified which solvent was used for each test substance. However, the study indicated that appropriate solvent controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods presented in the study report were described adequately; however, methods associated with cytological and ATP analyses were cited to another publication.
	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 was not described how volatile test substances were handled. This is considered to have substantially impacted 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	The methods and Table 1 indicate that the test substance was evaluated at 1000 ug/mL.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and considered appropriate for the study type (20 hr).
	Metric 12:	Exposure Route and Method	Low	× 1	3	The study report suggests that one dose was tested (prescreening) rather than at least two as recommended for similar study types.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Chinese hamster ovary (CHO) cells were utilized for this study. The identity, source, and culture methods for the CHO cells were reported. This cell line is routinely used for genotoxicity endpoints.

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Study Citation:	N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465
Data Type:	Inhibition of DNA and protein synthesis for CCl4
HERO ID:	626038

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The methods indicate that each experimental condition was conducted with n = 3 technical replicates, with n = 5 replicates for controls.; each experiment was conducted twice. Based on data presented in Table 1, it appears that at least 6 replicates were used for CCl4.
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 the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the 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	High	× 1	1	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	No statistical analysis was performed, and raw data were not provided to enable independent statistical analysis. The data shown for CCl4 in Table 1 (DNA synthesis) are shown as the percentage of control.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not reported.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were defined in the study report, and methods used for assessing cytotoxicity were adequately described (i.e., trypan dye exclusion).
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported for the 1000 ug/mL group only (Table 1); data were expressed as the percentage of the control (i.e., control data were not shown).
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

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Study Citation:	N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465
Data Type:	Inhibition of DNA and protein synthesis for CCl4
HERO ID:	626038

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[**] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 59: **In vitro evaluation results of Imperial Chemical Industries Ltd. 1976 for genotoxicity-bacterial reverse mutation**

Study Citation:	Imperial Chemical Industries Ltd. (1976). Mutagenicity testing with salmonella typhimurium strains on plates, of gases, liquids and solids for Imperial Chemical Industries Limited with attachments
Data Type:	Genotoxicity-bacterial reverse mutation
HERO ID:	4215890

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified; CASRN not reported.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test manufacturer of the substance was reported, but batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Authors reported the use of negative controls.
	Metric 5:	Positive Controls	High	× 2	2	Authors reported use of positive controls.
	Metric 6:	Assay Procedures	Low	× 1	3	Assay procedure was described with limited assay details.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	The QC part of this test criteria may not be applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Although exposure administration information was provided it is incomplete making it less consistent.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Authors reported three doses/concentrations of the test chemical.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration reported was lower than standard assays.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Although the number of exposure groups was sufficient, the higher and highest exposure concentrations were highly toxic.
	Metric 13:	Metabolic Activation	Low	× 1	3	The authors reported the use of metabolic activation, however, the methods lack the details of the protocol.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The authors reported the strain types, their properties and the description of the test model, but provided limited details.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The authors mentioned the adaptation of a a standard assay method. However, they did not provide details of the number and replicates used per study group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The reporting was incomplete and it was unclear whether methods were sensitive for the outcome of interest.

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Study Citation:	Imperial Chemical Industries Ltd. (1976). Mutagenicity testing with salmonella typhimurium strains on plates, of gases, liquids and solids for Imperial Chemical Industries Limited with attachments
Data Type:	Genotoxicity-bacterial reverse mutation
HERO ID:	4215890

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The authors did not provide specifics about the execution of the study protocol.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Details regarding sampling of outcomes were not fully reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for this study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Details about the number of organisms used per group were not reported. These deficiencies are likely to have a substantial impact on results.
	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	Although data is available for calculations, the high toxicity seen at two higher doses might impact the analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	High toxicity makes the interpretation of data difficult.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Although the endpoints of cytotoxicity were defined, the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Study authors reported data for all exposure groups.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.4	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 60: **Animal toxicity evaluation results of Doolittle et al., 1987 study on DNA replication and repair**

Study Citation:	D. J. Doolittle, G. Muller, H. E. Scribner (1987). Relationship between hepatotoxicity and induction of replicative DNA synthesis following single or multiple doses of carbon tetrachloride Journal of Toxicology and Environmental Health, 22(1,1), 63-78
Data Type:	DNA replication and repair
HERO ID:	194155

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was manufactured by Aldrich Chemical Co.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was identified as >99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative controls were administered.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcomes assessed.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance was dissolved just prior to dosing.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Test substance was administered consistently and appropriately.
	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 and duration were appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 groups for DNA repair; 6 groups for replication; liver toxicity was assessed.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were reported and appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Health status of mice was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some husbandry conditions were not reported (i.e. temperature, humidity, light/dark cycles).
	Metric 15:	Number per Group	High	× 1	1	3-6 mice / group were studied.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for outcomes (30-50 cells for UDS; 1000 cells for replication).
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were coded and scored without knowledge of treatment.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control did not elicit response.
Domain 6: Confounding / Variable Control						

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Study Citation:	D. J. Doolittle, G. Muller, H. E. Scribner (1987). Relationship between hepatotoxicity and induction of replicative DNA synthesis following single or multiple doses of carbon tetrachloride Journal of Toxicology and Environmental Health, 22(1,1), 63-78
Data Type:	DNA replication and repair
HERO ID:	194155

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weights and food/water intake was not reported across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data was presented as mean ± standard deviation
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported without ambiguity in figures and text.
Overall Quality Determination[‡]			High		1.4	
Extracted			Yes			

[⋆] MWF = Metric Weighting Factor.

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

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

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

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

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

Table 61: **Animal toxicity evaluation results of Nath et al., 1990 study on DNA synthesis**

Study Citation:	R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compunds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357
Data Type:	DNA synthesis
HERO ID:	6146

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle controls were used (same injection volume).
	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	Preparation in corn oil was described. Storage was not reported; however, only a single injection was used.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Information was provided to allow calculation of dose (% v/v, ml/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose was adequate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose group; level was not justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported. The test animal was obtained from a commercial source. Body weight and health status were not reported. Mice were described as retired breeders (10-12 months old).
	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	3-4/group was adequate for the outcome of interest.
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	High	× 1	1	Outcome was 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.

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Study Citation:	R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compunds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357
Data Type:	DNA synthesis
HERO ID:	6146

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 20: Negative Control Response	High	× 1	1	Negative control response appears 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	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 described and appropriate.
	Metric 24: Reporting of Data	High	× 2	2	Data were fully reported across timepoints.
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 62: **Animal toxicity evaluation results of Wacker et al., 2001 study on HNE DNA adduct quantitation**

Study Citation:	M. Wacker, P. Wanek, E. Eder (2001). Detection of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats Chemico-Biological Interactions, 137(3,3), 269-283
Data Type:	HNE DNA adduct quantitation after ip CCl4
HERO ID:	194416

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material was reported by name.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source was reported. Lot/batch was not reported, but the composition is not expected to vary.
	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	Concurrent untreated control was reported. No vehicle was indicated for CCl4.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control 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.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	It is unclear whether test substance was diluted in a vehicle prior to injection. Injection volume was reported to be small (50 uL).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was inferred from text to be consistent across study .
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose were clearly reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and were adequate for the study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Single dose group was adequate for the outcome; dose level was not justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was appropriate for the test .
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal source and characteristics were reported and appropriate.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animals husbandry was reported and adequate.
	Metric 15:	Number per Group	Medium	× 1	2	Number of animals per group was inferred from the text and appeared adequate for statistical .
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was inferred to be carried out .

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Study Citation:	M. Wacker, P. Wanek, E. Eder (2001). Detection of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats Chemico-Biological Interactions, 137(3,3), 269-283
Data Type:	HNE DNA adduct quantitation after ip CCl4
HERO ID:	194416

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric was not applicable for the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type.
	Metric 20:	Negative Control Response	Medium	× 1	2	Negative controls appeared to respond 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 across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and were appropriate for the data set.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes.
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 63: **Animal toxicity evaluation results of Chung et al., 2000 study on HNE DNA adducts in rat liver**

Study Citation:	F. L. Chung, R. G. Nath, J. Ocando, A. Nishikawa, L. Zhang (2000). Deoxyguanosine adducts of t-4-hydroxy-2-nonal are endogenous DNA lesions in rodents and humans: detection and potential sources Cancer Research, 60(6,6), 1507-1511					
Data Type:	HNE DNA adducts in rat liver					
HERO ID:	194418					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was clearly identified as CCl4.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance 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	High	× 2	2	Concurrent vehicle-only (olive oil) treated animals served as a negative control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	The allocation of animals was inferred from the text to be random. The study indicated that 15 rats were divided into 3 groups of 5 animals.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was inferred (i.e., dissolved in olive oil), but storage was not reported (considered unlikely to affect results owing to the short-term nature of the study).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups (single i.p. dose).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The dose was reported without ambiguity (3.2 g/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The duration of exposure was appropriate to detect/identify adducts (24 and 72 hours following a single exposure).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The rationale for the selected dose was provided. This dose used had been shown to induce lipid peroxidation in rats.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was clearly specified (single intraperitoneal injection).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The study indicated that 13-week-old male F344 rats were used. The source of the rats and their initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not sufficiently reported to evaluate if differences occurred between control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate for the outcome of interest (5/group).
Domain 5: Outcome Assessment						

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Study Citation:	F. L. Chung, R. G. Nath, J. Ocando, A. Nishikawa, L. Zhang (2000). Deoxyguanosine adducts of t-4-hydroxy-2-nonal are endogenous DNA lesions in rodents and humans: detection and potential sources Cancer Research, 60(6,6), 1507-1511
Data Type:	HNE DNA adducts in rat liver
HERO ID:	194418

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was described in adequate detail.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The outcome assessment appeared to be consistent across study groups, except that control animals were sacrificed with the 24 hour time group (no control for the 72 hour time group).
	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.
	Metric 20:	Negative Control Response	Medium	× 1	2	Negative control responses were appropriate but had limitations: adducts are also generated endogenously.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information pertaining to confounding variables in test design 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						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were not reported, however data and statistical results were provided and appeared appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes.
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 64: **Animal toxicity evaluation results of Wang et al., 1995 study on MDA DNA adducts in hamster liver and kidney**

Study Citation:	M. Y. Wang, J. G. Liehr (1995). Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis Archives of Biochemistry and Biophysics, 316(1,1), 38-46
Data Type:	MDA DNA adducts in hamster liver and kidney
HERO ID:	194420

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source was reported as Sigma Co. Lot/batch was not reported, but composition is not expected to vary.
	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 vehicle controls (corn oil) served as negative control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable 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	Test substance was inferred to be prepared by dilution in corn oil, storage was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in ml/kg and can be converted.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure followed by 4 hours before sacrifice was reported and appropriate for the outcome.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Exposure groups and spacing were not justified by the authors but appeared appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were appropriate for the test .
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, age and commercial source were identified. BOdy weight and health status were not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not sufficiently reported.
	Metric 15:	Number per Group	High	× 1	1	4/group was sufficient for the outcome of .
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was inferred from text to be carried out consistently.
	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.

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Study Citation:	M. Y. Wang, J. G. Liehr (1995). Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis Archives of Biochemistry and Biophysics, 316(1,1), 38-46
Data Type:	MDA DNA adducts in hamster liver and kidney
HERO ID:	194420

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 20:	Negative Control Response	Medium	× 1	2	Negative control responses were reported and appeared appropriate.
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 across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data shown for all .
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 65: **Animal toxicity evaluation results of Chaudhary et al., 1994 study on M1G -deoxyribose and lipid peroxidation**

Study Citation:	A. K. Chaudhary, M. Nokubo, G. R. Reddy, S. N. Yeola, J. D. Morrow, L. J. Blair IA: Marnett (1994). Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver Science, 265(5178,5178), 1580-1582
Data Type:	M1G -deoxyribose, lipid peroxidation
HERO ID:	194422

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by established nomenclature as CCL4; no CASRN was provided.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test material 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	High	× 2	2	A concurrent vehicle (corn oil) control was used
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not generally used 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	Limited information on preparation (CCL4 was administered in corn oil) and no information on storage were provided in this study. Storage conditions are unlikely to affect the results given the short duration (single exposure) of the study
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Consistency of gavage volumes (0.9mL/kg) across groups was inferred from the text, and the volume was appropriate. Time of day was not reported but unlikely to substantially impact the results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The single dose (0.1 mg/kg bw) was clearly reported
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The frequency/duration (single dose) was clearly reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Justification for the dose was not reported however, a response was observed. The single dose was acceptable for the outcome of interest
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route (gavage) was appropriate for the test substance, the study type, and the outcome of interest.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal species and strain (Sprague-Dawley rats) were reported, but source, sex, age, and starting body weights were not provided.
	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/group (n = 5) was reported and appropriate for the outcome of interest.

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Study Citation:	A. K. Chaudhary, M. Nokubo, G. R. Reddy, S. N. Yeola, J. D. Morrow, L. J. Blair IA: Marnett (1994). Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver Science, 265(5178,5178), 1580-1582
Data Type:	M1G -deoxyribose, lipid peroxidation
HERO ID:	194422

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Methodological details on how M1G residues were assessed were omitted.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The consistency of outcome assessment between the treatment and control group cannot be determined due to lack of methodological details.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Details regarding sampling (extractions from liver) were not reported, but are unlikely to have a substantial impact on results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable; the outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response (M1G residue concentration) of the control animals was reported quantitatively and appeared appropriate .
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, health status, and food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The statistical method used to compare M1G residue levels was reported and appropriate (Wilcoxon rank sum test).
	Metric 24:	Reporting of Data	Medium	× 2	4	M1G residue means were provided with an unspecified measure of error (SD or SEM).
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 66: **Animal toxicity evaluation results of Schwarz et al., 1979 study on alkaline elution assay in NMRI mice**

Study Citation:	M. Schwarz, J. Hummel, K. E. Appel, R. Rickart, W. Kunz (1979). DNA damage induced in vivo evaluated with a non-radioactive alkaline elution technique Cancer Letters, 6(4-5,4-5), 221-226
Data Type:	alkaline elution assay in NMRI mice
HERO ID:	194425

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as CCl4.
	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 vehicle controls were used.
	Metric 5:	Positive Controls	High	× 1	1	Several compounds typically used as positive controls were studied (e.g., DMN, MMS, AAF).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was inferred to be diluted in vehicle. Storage was not .
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration is inferred to be consistent across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in table 1 clearly in ml/kg and can be converted.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and .
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of exposure groups and spacing were not justified by the authors but apapeared appropriate for the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not .
	Metric 15:	Number per Group	High	× 1	1	Number of animals was reported in table 1 (n=5) and is sufficient for .
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was partially reported, is commonly used, and was sensitive for the outcome of .
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	It was inferred that the outcome assessment was carried out .
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type

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Study Citation:	M. Schwarz, J. Hummel, K. E. Appel, R. Rickart, W. Kunz (1979). DNA damage induced in vivo evaluated with a non-radioactive alkaline elution technique Cancer Letters, 6(4-5,4-5), 221-226
Data Type:	alkaline elution assay in NMRI mice
HERO ID:	194425

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
	Metric 20:	Negative Control Response	Medium	× 1	2	Negative control response was reported, however it is is unclear if all vehicle controls (and all time points) were combined in table 1.
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 across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was not described, however data reported was sufficient for independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and .
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 67: **Animal toxicity evaluation results of Stewart et al., 1981 study on rat liver DNA damage**

Study Citation:	B. W. Stewart (1981). Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA in vivo Cancer Research, 41(8,8), 3228-3243
Data Type:	Rat liver DNA damage
HERO ID:	194464

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle controls were described in table legend.
	Metric 5:	Positive Controls	High	× 1	1	Several compounds that could be considered positive controls were assessed (e.g., DEN, BaP, MMS).
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was diluted in corn oil, as inferred from table 1 legend. Storage was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was assumed to be consistent.
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Doses were reported in a range 200-800 mg/kg in table 1, not specified further.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency (single dose) and duration (4 or 24 hour) were reported
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Number of groups was not reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method are appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal characteristics (sex and strain) were partially reported. Source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not sufficiently reported.
	Metric 15:	Number per Group	Low	× 1	3	Number of animals was reported as pairs.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Outcome assessment methodology appears adequate, but is not commonly used (caffeine elution).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was inferred through text to be carried out consistently across test 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.

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Study Citation: B. W. Stewart (1981). Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA in vivo Cancer Research, 41(8,8), 3228-3243
Data Type: Rat liver DNA damage
HERO ID: 194464

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 20: Negative Control Response	Unacceptable	× 1	4	Negative control responses were not reported.
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 across groups.
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	Unacceptable	× 1	4	Statistics were not reported and data was not sufficient for an independent statistical analysis
	Metric 24: Reporting of Data	Unacceptable	× 2	8	Data was reported qualitatively in table 2 for each timepoint, but not by dose.
Overall Quality Determination[‡]		Unacceptable[⋆⋆]		2.7	
Extracted		No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 68: **Animal toxicity evaluation results of Suzuki et al., 1997 study on mouse micronucleus assay**

Study Citation:	H. Suzuki, N. Hirano, C. Watanabe, Y. Tarumoto (1997). Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood Mutation Research, 394(1-3,1-3), 77-80					
Data Type:	Mouse micronucleus assay for CCl4					
HERO ID:	194473					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as CCl4 and by CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Wako Pure chemical). Although a lot/batch number was not reported, 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	Concurrent negative (vehicle-only) control groups were reported (assays in bone marrow and peripheral blood). For the peripheral blood micronucleus assay, negative controls were included for each time point.
	Metric 5:	Positive Controls	Medium	× 1	2	Positive control groups were used (mitomycin C) and positive responses were observed. Positive controls were used at each time point (peripheral blood assay); however, a positive control was not used for the double-dosing experiment (bone marrow assay).
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (i.e., dissolved in olive oil). Although storage was not reported, this omission is unlikely to impact the results (short-term study).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was inferred to be consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported clearly in Tables 1 (bone marrow test) and 2 (peripheral blood test).
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	The exposure frequency/duration were reported and were appropriate for the outcome of interest (single- or double-dosing for the gavage bone marrow test and single i.p. injection for the peripheral blood test). The time between dosing for the gavage experiment (double-dosing protocol) was not explicitly specified.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Doses were justified with a preliminary acute study (gavage bone marrow test) or based on a previous acute lethality test (i.p. peripheral blood test); the number of groups was consistent with studies of this type.

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Study Citation: H. Suzuki, N. Hirano, C. Watanabe, Y. Tarumoto (1997). Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood Mutation Research, 394(1-3,1-3), 77-80
Data Type: Mouse micronucleus assay for CCl4
HERO ID: 194473

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route for the bone marrow test was appropriate for the study type (gavage); the route for the peripheral blood test (i.p. injection) is not recommended by study type (not relevant to human exposure); no justification was reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were briefly described; animals were obtained from a commercial source. Initial body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions (temperature, humidity) were reported and were adequate and similar for all groups. Light/dark cycle was not reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported (n =5 per group and/or time point) and appropriate for the outcome of interest.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	In the bone marrow test, the outcome was assessed at only one time point after dosing a single time (two time points are recommended). In the peripheral blood test (single i..p. dose), at least two time points are recommended and were used (48 and 72 hours); the test also evaluated the outcome for peripheral cells at 24 hours (not recommended by study type).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was inferred to be carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling was (1000 erythrocytes per animal) and was adequate to evaluate the outcome of interest for the bone marrow assay and slightly less than recommended for the peripheral blood.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding of assessors was not reported and was not needed as outcomes assessed via Giemsa analysis were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on confounding variables in test design or procedure were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No differences in health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analyses were performed; methods were cited to another publication.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes.

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Study Citation:	H. Suzuki, N. Hirano, C. Watanabe, Y. Tarumoto (1997). Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood Mutation Research, 394(1-3,1-3), 77-80
Data Type:	Mouse micronucleus assay for CCl4
HERO ID:	194473

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Overall Quality Determination[‡]		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 69: **Animal toxicity evaluation results of Takahashi et al., 1998 study on DNA adducts**

Study Citation:	S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment Toxicologic Pathology, 26(2,2), 247-252
Data Type:	DNA adducts for CCl4
HERO ID:	194478

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified by name (carbon tetrachloride).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (a manufacturer) was identified. 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 >99.8%; therefore, observed effects are likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a negative control group (clearly marked as a vehicle-only control).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not generally used for the study/outcome 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	Preparation of the test substance was described (i.e., dissolved in olive oil), but storage was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Deficiencies in reporting of administered doses occurred (i.e., 5 mL/kg and no information on animal body weight).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were administered a single gavage dose of the test substance (appropriate for the outcome of interest).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Only one dose was used to evaluate DNA adduct formation; however, this dose was sufficient to elicit a positive response at two time points. The study noted that there was an extensive database for hepatotoxicity related to CCl4 , and that histopathological liver effects were seen after a single 1 mL/kg dose (but the dose used in this study was 5 mL/kg).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance (oral gavage).
Domain 4: Test Organism						

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Study Citation:	S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment Toxicologic Pathology, 26(2,2), 247-252
Data Type:	DNA adducts for CCl4
HERO ID:	194478

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Minor uncertainties in the reporting of test animal characteristics (health status and starting body weight) are unlikely to have a substantial impact on results. The test animals were obtained from a commercial source was appropriate for the outcome of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals per study group was lower than the typical number used (3 males/time point), but sufficient for statistical analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was briefly described, and partially cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was evaluated 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.
	Metric 20:	Negative Control Response		× 1	NA	The biological responses of the negative control groups were adequate (low incidence of adducts).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Information on confounding variables 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	Medium	× 1	2	Statistical analysis was not described (e.g., test used); but statistically analyzed data were reported. From the data presented in Figure 2 (and n = 3), means and measures of variance could be estimated.
	Metric 24:	Reporting of Data	High	× 2	2	Data is presented in Figure 2 (control and treatment groups at each time point).
Overall Quality Determination‡			Medium		0.0	
Extracted			Yes			

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Study Citation:	S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment Toxicologic Pathology, 26(2,2), 247-252
Data Type:	DNA adducts for CCl4
HERO ID:	194478

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 70: **Animal toxicity evaluation results of Tombolan et al., 1999 study on mitogenic and regenerative cell proliferation**

Study Citation:	F. Tombolan, D. Renault, D. Brault, M. Guffroy, F. Perin, V. Thybaud (1999). Effect of mitogenic or regenerative cell proliferation on lacz mutant frequency in the liver of MutaTMMice treated with 5, 9-dimethyldibenzo[c,g]carbazole Carcinogenesis, 20(7,7), 1357-1362
Data Type:	Mitogenic and regenerative cell proliferation
HERO ID:	194500

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the substance was identified as Prolabo (Paris, France).
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative control group was included (vehicle only), however mice in control group were sacrificed on day 1 and 7 and pooled. Experimental group were sacrificed on day 1, 2, 3, 4 and 7. This is unlikely to have a substantial impact on results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive responses were verified in small intestine tissue within the same animal and/or were elicted with other substances run. Results from positive control (small intestine) samples were not reported.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of test animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of test substance was reported. Storage of test substance was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose concentration was reported to be 80 mg/kg.
	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	High	× 1	1	Only one dose (80mg/kg) was used in this study therefore dose-responses were not reported. However, positive results were obtained from this dose, so it was considered to be an adequate dose for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was appropriate (oral gavage).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strains, sex, age, and commercial source were reported. Health status and beginning body weights were not provided. Details regarding the unique genetic feature (bacterial Lacz reporter gene) of the Muta(TM)Mice were described.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported.

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Study Citation:	F. Tombolan, D. Renault, D. Brault, M. Guffroy, F. Perin, V. Thybaud (1999). Effect of mitogenic or regenerative cell proliferation on lacz mutant frequency in the liver of MutaTMMice treated with 5, 9-dimethyldibenzo[c,g]carbazole Carcinogenesis, 20(7,7), 1357-1362
Data Type:	Mitogenic and regenerative cell proliferation
HERO ID:	194500

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	For CD2F1 mice studies, n = 4 mice/treatment group. For Muta(TM)Mice studies, n = 5 mice/treatment group. For negative controls, n = 2-3 mice.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome methodology was partially reported and cited elsewhere.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was reported and consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	BrdU-immunopositive nuclei: 3000-35000 hepatocellular nuclei scored. Mutant frequency determination: at least 200,000 plaque-forming units by animal were recorded.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not necessary for these studies.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights and food/water intake were not reported however it is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes unrelated to test substance were not reported although it unlikely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed by Student's t-test and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes in Figures/ Tables and text.
Overall Quality Determination[‡]			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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 71: **Animal toxicity evaluation results of Mirsalis et al., 1980 study on in vivo UDS**

Study Citation:	J. C. Mirsalis, B. E. Butterworth (1980). Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens Carcinogenesis, 1(7,7), 621-625
Data Type:	In vivo UDS
HERO ID:	194512

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Source was identified as Fisher Scientific Co.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative control was included (vehicle).
	Metric 5:	Positive Controls	High	× 1	1	Dimethylnitrosamine and acetylaminofluorene were both utilized as positive control substances to test the novel method of assessing UDS ex vivo.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance was prepared in corn oil. Storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across treatment groups (negative controls and two doses of CCl4).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses are reported as 10 and 100 mg/kg.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure was reported as 2 hr. This sampling time after test substance administration appeared to be appropriate due to the positive response from DMN and AAF at this timepoint. However, current standards recommend a longer timepoint, such as 12-16 hours; it is unclear why DMN- and AAF-treated animals were sampled at 12, 24, and 48 hours, while CCl4-treated animals were not.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Only 2 doses used. Not clear if highest dose was high enough to elicit response. There was no justification for the selection of the doses. The highest dose tested should produce signs of toxicity such that higher doses would be expected to result in mortality. There was no indication that the test animals administered the highest dose of CCl4 (100 mg/kg) exhibited signs of toxicity.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route of exposure was appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Age and health status are not reported, it is unlikely to have a substantial impact on results

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Study Citation:	J. C. Mirsalis, B. E. Butterworth (1980). Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens Carcinogenesis, 1(7,7), 621-625
Data Type:	In vivo UDS
HERO ID:	194512

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions are not sufficiently reported.
	Metric 15:	Number per Group	Low	× 1	3	It appears that each "experiment" referred to in Table II refers to a single animal. Thus, for 10 mg/kg, n = 1. For 100 mg/kg, n = 2. The number per group is considered to be lacking.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methodology is partially reported and cited elsewhere (Bermudez, 1979).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	50 cells were scored for each of 3 slides per group, which is in line with current guidelines.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Automated measurements were used.
	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 in test design reported. Initial body weights were 200-250 g
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not conducted. Data are presented as mean ± SE and SD. However, independent statistical analysis could not be completed appropriately from the mean, SD, and n because the study conflated slide-to-slide variability with experiment-to-experiment variability. The data appeared to be inappropriately pooled (using n = 300 rather than n = 2) and thus resulted in reporting smaller standard deviations than reality.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported in Table II.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		1.7	
Extracted			No			

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Study Citation:	J. C. Mirsalis, B. E. Butterworth (1980). Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens Carcinogenesis, 1(7,7), 621-625
Data Type:	In vivo UDS
HERO ID:	194512

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 72: **Animal toxicity evaluation results of Mirsalis et al., 1989 study on DNA replication and UDS in hepatocytes**

Study Citation:	J. C. Mirsalis, C. K. Tyson, B. E. Butterworth (1982). Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay Environmental and Molecular Mutagenesis, 4(5,5), 553-562					
Data Type:	DNA replication and UDS in hepatocytes					
HERO ID:	10063					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was identified as Fisher Scientific Co.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was identified as ACS grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative controls were included.
	Metric 5:	Positive Controls	High	× 1	1	Positive responses were elicited by 2-AAF and MMS run concurrently.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in corn oil. Time frame from when solution was made until administered is not given, unclear if storage was necessary.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in Table I and II (40 and 400 mg/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequencies and duration were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Ability of CCl4 to induce DNA replication was only performed at one dose (400mg/kg). DNA repair (UDS) was evaluated at two doses (40 and 400 mg/kg)
	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	High	× 2	2	Species, strain, sex, initial body weight, health status and commercial source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Number of animals/groups was reported to be 2-4 in Figure 1 and Table I and II.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methodology for UDS was described, however assessment methodology for DNA replication was not described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across groups..

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Study Citation:	J. C. Mirsalis, C. K. Tyson, B. E. Butterworth (1982). Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay Environmental and Molecular Mutagenesis, 4(5,5), 553-562
Data Type:	DNA replication and UDS in hepatocytes
HERO ID:	10063

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	High	× 1	1	Random selection of 50 morphological unaltered cells were counted.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Study used an ARTEK Model 880 colony counter and data were fed directly into a Digital Equipment Corp Vax11/780 computer.
	Metric 20:	Negative Control Response	High	× 1	1	Responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weights and food/water intake were not reported for each study group.
	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	Data is presented at mean± SE.
	Metric 24:	Reporting of Data	Medium	× 2	4	It is unclear if data points were not reported due to lack of details in methods. Figure 1 shows time point for 24hr and 48 hr that are not reported in Table II. The dose used to generate Figure 1 was also not reported. It is not clear if other doses/ timepoints were used to investigate DNA replication (Table 1).
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: **Animal toxicity evaluation results of Morita et al., 1997 for micronucleus assay**

Study Citation:	T. Morita, N. Asano, T. Awogi, Y. F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS Mutation Research, 389(1,1), 3-122
Data Type:	Micronucleus assay for CCl4
HERO ID:	194532

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 (carbon tetrachloride). A CASRN (56-23-5) was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (a manufacturer) was reported. A lot number (Lot # SAK7972) was provided.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99.8%); therefore, effects are likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (vehicle-only) control group for the bone marrow assay, and a sample before treatment (0 hours) as a negative control in the peripheral blood assay.
	Metric 5:	Positive Controls	Medium	× 1	2	The study authors reported using a positive control (mitomycin C) in the micronucleus assays. It was indicated that positive responses were observed, but data were not shown.
	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 was dissolved in olive oil. Lack of reporting with respect to storage conditions is not likely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in Table 5.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration were reported (i.e., the number/spacing of treatments) and were appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (3 doses [plus negative control for the bone marrow assay]) was reported. Doses were selected based on the outcome of preliminary dose-finding tests.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The bone marrow assay was performed by oral gavage; the peripheral blood assay was performed by i.p. injection (not considered a relevant route of human exposure).
Domain 4: Test Organism						

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Study Citation:	T. Morita, N. Asano, T. Awogi, Y. F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS Mutation Research, 389(1,1), 3-122
Data Type:	Micronucleus assay for CCl4
HERO ID:	194532

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animals was not reported. The strain, sex, and age of the mice used in the study were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group (5 males) was reported, appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Details regarding outcome assessment methodology were limited. In general, the timing of assessments were sensitive for the outcome of interest (two sampling times in the recommended time frame for the peripheral blood assay; one sampling time in the recommended time frame for the bone marrow assay after two gavage doses). The single gavage dose bone marrow assay only one sampling time (two are recommended).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Although limited details are reported, the data tables indicate that outcomes were assessed consistently across study groups (at the same times after initial exposure).
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling was reported (1000 polychromatic erythrocytes or reticulocytes/animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type (blinding not reported).
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the negative control group) were adequate (low incidences of micronucleus formation). The study authors noted that historical control data were not always available, and that control data were judged subjectively.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Confounding variables in test design and procedures were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data are presented as mean ±SD in Table 5. Statistical analyses were performed. Micronucleus frequency in treated groups was compared to concurrent controls using a conditional binomial test; the does-response was evaluated using a Cochran-Armitage trend test.
	Metric 24:	Reporting of Data	High	× 2	2	Data for outcomes is presented in Table 5 (by assay, time point. exposure group).

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Study Citation:	T. Morita, N. Asano, T. Awogi, Y. F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS Mutation Research, 389(1,1), 3-122
Data Type:	Micronucleus assay for CCl4
HERO ID:	194532

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Overall Quality Determination[‡]		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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: **Animal toxicity evaluation results of Uryvaeva et al., 1995 study on a micronucleus assay**

Study Citation:	I. V. Uryvaeva, G. V. Delone (1995). An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study Mutation Research, 334(1,1), 71-80
Data Type:	Micronucleus assay for CCl4
HERO ID:	194598

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 (carbon tetrachloride).
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance 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	Unacceptable	× 2	8	CCl4-treated mice were compared to mice with partial hepatectomy. Negative controls were not employed.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. The aim of the study was to compare micronuclei formation (and its age dependence) following mitotic stimulation via CCl4 or partial hepatectomy.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	There was no mention of the method and equipment used to generate the test substance.
	Metric 8:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., methods for generating atmosphere in inhalation studies) were not reported. In addition, animals were evaluated at 2.5 months after partial hepaectomy [PH] or CCl4 treatment only; at the other time points, there was only one condition or the other (PH at 5 months and CCl4 treatment at 7 months).
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Actual concentrations and/or analytical methods were not reported. The study indicated the range of exposure (0.05 to 0.1 mL).
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	The duration of exposure was reported (15 minutes). The duration of the study was likely not sufficient to induce micronuclei induction. The study was intended to evaluate the ability of CCl4 to induce mitosis in the liver (and was based on the assumption that the test substance is non-genotoxic).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	A single dose level was used to characterize the age-dependence of micronuclei induction in hepatocytes. The dose was adequate for that purpose, but the concentration was not likely high enough for the purpose of evaluating the genotoxicity of CCl4.

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Study Citation: I. V. Uryvaeva, G. V. Delone (1995). An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study Mutation Research, 334(1,1), 71-80
Data Type: Micronucleus assay for CCl4
HERO ID: 194598

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Low	× 1	3	Animals were exposed to the test substance in a 5L sealed box; distribution of the the substance in the whole-body chamber was not reported (not clear if dynamic).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Minor uncertainties in the reporting of test animal characteristics (health status, starting body weight) are unlikely to have a substantial impact on results. The test animals were obtained from a nursery farm, and the test species was an appropriate animal model.
	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	It appears that there were at least 5 males/group, which is adequate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methodology related to micronucleus examination was described and partially cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes appeared to be assessed consistently after exposure.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	The study indicated that 1000 (animals aged 5 and 7 months) to 2000 cells/animal (animals aged 2.5 months) were screened for micronuclei.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the PH group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions of study groups were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were presented as means +/- SD. Data were presented in a form amenable to independent statistical analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.4	
Extracted			No			

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Study Citation:	I. V. Uryvaeva, G. V. Delone (1995). An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study Mutation Research, 334(1,1), 71-80
Data Type:	Micronucleus assay for CCl4
HERO ID:	194598

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 75: **Animal toxicity evaluation results of Van Goethem et al., 1995 study on a micronucleus assay**

Study Citation:	F. Van Goethem, J. de Stoppelaar, B. Hoebee, M. Kirsch-Volders (1995). Identification of clastogenic and/or aneugenic events during the preneoplastic stages of experimental rat hepatocarcinogenicity by fluorescence in site hybridization Carcinogenesis, 16(8,8), 1825-1834					
Data Type:	Micronucleus assay for CCl4					
HERO ID:	194600					
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 CCl4.
	Metric 2:	Test Substance Source	High	× 1	1	The source of test substance (a manufacturer) 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	Low	× 1	3	Purity/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study reported using a negative control group. However, it is likely that an untreated control (rather than a vehicle-only control) was used (but this is not entirely clear).
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used, but treatment-related positive responses were observed (the test is capable of detecting a positive response).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was described, however details on storage conditions are not. It is unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across the study group.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The dose was reported as 2mL/kg. A dose in mg/kg was not provided, and only a range of initial body weights (i.e., 200 to 220 g) was reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration (i.e., single gavage dose) were reported and were appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups was reported (1 plus controls). The dose was presumably selected based on previous studies. The number of dose groups is fewer than that recommended by study type; however, the aim of this study was to evaluate micronuclei formation during carcinogenesis induced by initiation, promotion, and CCl4 treatment. The CCl4 only treatment group was a preliminary/control experiment for this treatment protocol.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was reported and appropriate for the study type.
Domain 4: Test Organism						

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Study Citation:	F. Van Goethem, J. de Stoppelaar, B. Hoebee, M. Kirsch-Volders (1995). Identification of clastogenic and/or aneugenic events during the preneoplastic stages of experimental rat hepatocarcinogenicity by fluorescence in site hybridization Carcinogenesis, 16(8,8), 1825-1834
Data Type:	Micronucleus assay for CCl4
HERO ID:	194600

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The strain, age, and weights (range) of the test species were reported. Minor uncertainties in the reporting of test animal characteristics (e.g., health status) are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some husbandry conditions were reported (standard conditions with 12 hour light/dark schedule). Minor uncertainties (temperature and humidity) are not expected to impact the study results.
	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 (3 males/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment were partially described and cited elsewhere. The outcome was assessed only 72 hours after treatment (at least two time points are recommended after a single exposure).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate (about 4000 hepatocytes/animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to the study type (blinding not reported).
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was adequate (low incidence of micronuclei; mean = 1.5%).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions (e.g., food/water intake) were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed for data related to CCl4 treatment alone (p-values were provided in the text). The data (Table 1) were presented as means +/- standard deviation for n =3 animals, which is also amenable to independent analyses.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination[‡]			Medium		1.8	
Extracted			Yes			

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Study Citation:	F. Van Goethem, J. de Stoppelaar, B. Hoebee, M. Kirsch-Volders (1995). Identification of clastogenic and/or aneugenic events during the preneoplastic stages of experimental rat hepatocarcinogenicity by fluorescence in site hybridization Carcinogenesis, 16(8,8), 1825-1834
Data Type:	Micronucleus assay for CCl4
HERO ID:	194600

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 76: **Animal toxicity evaluation results of Van Goethem et al., 1993 study on a micronucleus assay**

Study Citation:	F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis Carcinogenesis, 14(11,11), 2397-2406
Data Type:	Micronucleus assay for CCl4
HERO ID:	194601

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 (carbon tetrachloride).
	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 specified, the test substance is not expected to vary in composition.
	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	Low	× 2	6	The study reported using concurrent negative control groups. There were CCl4 and CCl4+NaCl treatment groups, which were compared to control (presumably untreated but not clearly specified) and NaCl-treated control groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used, but treatment-related positive responses were observed (the test is capable of detecting a positive response).
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported; the study only indicated that animals were divided into 10 groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was described, however details on storage conditions are not. It is unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The dose was reported as 2mL/kg. A dose in mg/kg was not provided, and only a range of initial body weights (i.e., 200 to 220 g) was reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration (i.e., single gavage dose) were reported and were appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups was reported (1 plus controls). The dose was presumably selected based on previous studies. The number of dose groups is fewer than that recommended by study type; however, the aim of this study was to evaluate micronuclei formation during carcinogenesis induced by initiation, promotion, and CCl4 treatment.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was reported and appropriate for the study type.
Domain 4: Test Organism						

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Study Citation:	F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis Carcinogenesis, 14(11,11), 2397-2406
Data Type:	Micronucleus assay for CCl4
HERO ID:	194601

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The strain, age, and weights (range) of the test species were reported. Minor uncertainties in the reporting of test animal characteristics (e.g., health status) are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some husbandry conditions were reported (standard conditions with 12 hour light/dark schedule). Minor uncertainties (temperature and humidity) are not expected to impact the study results.
	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 (2 males/group in CCl4, CCl4+NaCl, control, and NaCl only groups).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome methodology was partially described and partially cited to other publications (e.g., hepatocyte isolation). Although more than one time point for analyses after a single treatment is recommended, initial experiments were conducted to determine the optimal time point for scoring micronuclei.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was reported and adequate (at least 4000 hepatocytes scored per animal). .
	Metric 19:	Blinding of Assessors	High	× 1	1	This metric was not applicable to the study type (blinding not reported).
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological response of the negative control group was adequate (low incidence of micronuclei; mean = 0.2 to 1.0%).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions (e.g., food/water intake) were not reported. Inter-individual differences likely contributed to differences in response.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical methods used in the study were described; however, it does not appear that statistics were applied to data for CCl4 treatment alone (or +NaCl; there were only 2 animals per group.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data were reported by exposure group (individual animal). However, data were shown as % incidence (raw numbers of micronuclei not reported).
Overall Quality Determination[‡]			Medium		1.9	
Extracted			Yes			

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Study Citation:	F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis Carcinogenesis, 14(11,11), 2397-2406
Data Type:	Micronucleus assay for CCl4
HERO ID:	194601

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 77: **Animal toxicity evaluation results of Varela-Moreiras et al., 1995 study on DNA methylation**

Study Citation:	G. Varela-Moreiras, E. Alonso-Aperte, M. Rubio, M. Gasso, R. Deulofeu, L. Alvarez, J. Caballeria, J. Rodes, J. M. Mato (1995). Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment Hepatology, 22(4 Pt 1,4 Pt 1), 1310-1315
Data Type:	DNA methylation for CCl4
HERO ID:	194604

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 formula.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Control rats were untreated (not clear whether vehicle was 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	Unacceptable	× 1	4	No details were provided on preparation of test substance for injection or storage.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure appeared consistent.
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Dose was unknown. Injection volume was reported as mL/kg, but it is not clear whether CCl4 was dissolved in vehicle prior to injection.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Duration and frequency was appropriate for the study type (2x/week for 3 weeks).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single exposure group; dose not provided or justified.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Intraperitoneal injection was appropriate for the test substance, but not relevant to human exposure.
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	Medium	× 1	2	Temperature and light/dark cycle were reported and adequate. Humidity was not reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals (5/group) is appropriate for the outcome of interest.
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.

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Study Citation:	G. Varela-Moreiras, E. Alonso-Aperte, M. Rubio, M. Gasso, R. Deulofeu, L. Alvarez, J. Caballeria, J. Rodes, J. M. Mato (1995). Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment Hepatology, 22(4 Pt 1,4 Pt 1), 1310-1315
Data Type:	DNA methylation for CCl4
HERO ID:	194604

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	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 appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were adequately reported.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.2	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 78: **Animal toxicity evaluation results of Crebelli et al., 1999 study on a micronucleus assay**

Study Citation:	R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J. A. Vericat (1999). Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test Mutagenesis, 14(2,2), 207-215					
Data Type:	Micronucleus assay for CCl4					
HERO ID:	194679					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified by chemical name, structure, and CASRN in Table I (carbon tetrachloride, CCl4, 56-23-5).
	Metric 2:	Test Substance Source	High	× 1	1	Source for CCl4 was reported as C. Erba (appears to be a manufacturer).
	Metric 3:	Test Substance Purity	High	× 1	1	Purity for CCl4 was >99.5%; therefore, effects are likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative (vehicle-only) controls were used for each sex (Table III).
	Metric 5:	Positive Controls	High	× 1	1	Positive controls (5/sex) of 1 mg/kg colchicine and 2 mg/kg mitomycin C were used and produced positive responses (Table III). Substances were dissolved in water and administered to animals 24 hours prior to sacrifice.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported that animals were randomly allocated into treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was dissolved in the vehicle. Storage information was not required as a singular administered dose was used (short-term).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared consistent and included a single intraperitoneal injection to groups of 5 male and 5 female mice.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The doses were clearly reported in Table III (1500 and 3000 mg/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were appropriate. A single intraperitoneal injection was used.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Doses were selected based on the LD50 for the chemical. The reported intraperitoneal LD50 for CCl4 was 3750 mg/kg. Doses aimed to test ~40% and 70-80% of the intraperitoneal LD50 and were therefore set at 1500 and 3000 mg/kg.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Single intraperitoneal injections were used to administer the test substance to animals and was appropriate for the study type (but not environmentally relevant).
Domain 4: Test Organism						

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Study Citation:	R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J. A. Vericat (1999). Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test Mutagenesis, 14(2,2), 207-215
Data Type:	Micronucleus assay for CCl4
HERO ID:	194679

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Crl:CD-1 (ICR) BR mice (5/sex/dose) were used. Weights were approx. 25-35 g at the time of sacrifice. Animals were purchased from Charles River Italia S.p.A. (Calco, Lecco, Italy). Some details were lacking (health status and age) although are note expected to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported - 5 animals per cage at 22 degrees C with a relative humidity of 55% and a dark/light cycle of 12 hours. Animal care followed Directive 86/609/EEC.
	Metric 15:	Number per Group	High	× 1	1	The number of animals used was reported (5/sex/dose) and was appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was reported and was sensitive for the outcome of interest. Bone marrow was assessed at two time points after a single injection dose (as recommended by the study type).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes appeared to be assessed consistently across dose groups, as reported in Table III.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design. The study indicated that slides were scored by 1 to 2 experienced readers. A single reader read each set of slides to minimize bias.
	Metric 20:	Negative Control Response	High	× 1	1	Vehicle controls responded appropriately (results provided in Table III).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was conducted and appropriate (x^2 test and t-test). Additionally, sufficient data were provided to conduct independent analyses.
	Metric 24:	Reporting of Data	High	× 2	2	Results for each dose group and control group by sex were reported (Table III).
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J. A. Vericat (1999). Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test Mutagenesis, 14(2,2), 207-215
Data Type:	Micronucleus assay for CCl4
HERO ID:	194679

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 79: **Animal toxicity evaluation results of Curtis et al., 1968 study on CAs in mouse liver**

Study Citation:	H. J. Curtis, J. Tilley (1968). Chromosome aberrations in liver forced to regenerate by chemical or surgical methods Journals of Gerontology. Series A: Biological Sciences and Medical Sciences, 23(2,2), 140-141
Data Type:	CAs in mouse liver for CCl4
HERO ID:	194696

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	Low	× 1	3	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	CCl4-treated mice were compared to mice with partial hepatectomy. Negative controls were not employed.
	Metric 5:	Positive Controls	Unacceptable	× 1	4	Half of the animals were given X-rays; however, animals were also given CCl4 or underwent partial hepatectomy. No X-ray only group was used.
	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	it is inferred that a small volume of undiluted CCl4 was injected subcutaneously a single time (no vehicle was described).
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Minimal details were reported regarding exposure (72h prior to sacrifice).
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Assuming no dilution of test substance, dose can be calculated from mL/g bw.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Single injection may not be sufficient to show CAs at later time points (10 and 21 weeks of age; injected at 6 weeks)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose; not justified by the authors and not clear if dose was high enough.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route and method were appropriate for the test substance, but not environmentally relevant.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, age and commercial source were reported. Health status and body weight were not given.
	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	The number of animals used (5/group) is adequate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	The outcome assessment methods were cited to another publication (Stevenson & Curtis, 1961).

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Study Citation:	H. J. Curtis, J. Tilley (1968). Chromosome aberrations in liver forced to regenerate by chemical or surgical methods Journals of Gerontology. Series A: Biological Sciences and Medical Sciences, 23(2,2), 140-141
Data Type:	CAs in mouse liver for CCl4
HERO ID:	194696

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	The outcome assessment methods were cited to another publication (Stevenson & Curtis, 1961).
	Metric 18:	Sampling Adequacy	High	× 1	1	100 figures were scored per animal (500 per group).
	Metric 19:	Blinding of Assessors	High	× 1	1	Cells were scored blind.
	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 for each group.
	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	Means +/- SEM were provided in the figure.
	Metric 24:	Reporting of Data	Low	× 2	6	Data from the 72 hour sacrifice were not presented.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.5	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 80: **Animal toxicity evaluation results of Sasaki et al., 1998 for in vivo Comet assay**

Study Citation:	Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibasi, K. Yoshida, Q. Y. Su, N. Matsusaka, S. Tsuda (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 419(1-3,1-3), 13-20
Data Type:	In vivo Comet assay for CCl4
HERO ID:	38908

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative control groups were included (untreated controls). It was stated that previous studies from the laboratory showed no difference between untreated and concurrent vehicle (olive oil) treated controls.
	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 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	Preparation of the test substance was briefly reported. Storage of the test substance was not reported (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	The exposure was a single-dose administration, which is lower than the guideline suggests (at least two administrations). It is possible that this resulted in some false negatives across the various organs and timepoints tested.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing for CCl4 were appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, age, sex, and commercial source of the test animals were reported. The starting body weight range of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were adequate, appropriate, and consistent.

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Study Citation:	Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibasi, K. Yoshida, Q. Y. Su, N. Matsusaka, S. Tsuda (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 419(1-3,1-3), 13-20
Data Type:	In vivo Comet assay for CCl4
HERO ID:	38908

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this study design (n = 4).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Sampling was lacking for the outcome of interest (50 nuclei per organ per animal). Test guideline suggests 150 nuclei per animal.
	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 responses were observed in negative controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Starting body weights were not reported. Respiratory rates and food/water consumption were not reported, but this is appropriate given the study design.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No deaths or health outcomes unrelated to the exposure were reported for this experiment. Histopathological lesions of the liver, including necrosis, were observed at the two highest doses of CCl4 at 24 hours. These lesions were considered to be related to cytotoxicity induced by the test substance, as the changes were dose related.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were appropriately analyzed by one-way ANOVA with Dunnett's post-hoc test.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported adequately.
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 81: **Animal toxicity evaluation results of Higami et al., 2004 study on mouse and rat liver DNase and DNA fragmentation**

Study Citation:	Y. Higami, T. Tsuchiya, K. To, T. Chiba, H. Yamaza, D. Shiokawa, S. Tanuma, I. Shimokawa (2004). Expression of DNase gamma during Fas-independent apoptotic DNA fragmentation in rodent hepatocytes Cell and Tissue Research, 316(3,3), 403-407
Data Type:	
HERO ID:	194726

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substance identified by name
	Metric 2:	Test Substance Source	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 (vehicle) controls were reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for the study type
	Metric 6:	Randomized Allocation	Low	× 1	3	allocation of animals was not described
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	test substance was prepared in mineral oil; storage was not reported, but this is unlikely to affect results given that the study was short term in duration (single exposure)
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	exposure were administered consistently across groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose reported clearly as 0.25 g/100 g BW
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	frequency was a single administration, with sacrifice 8 or 24h post injection
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	number of exposure groups and dose spacing was not justified by study authors but appeared sufficient given that the expected response (necrosis with DNA fragmentation) was observed
	Metric 12:	Exposure Route and Method	High	× 1	1	exposure route was appropriate for the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	test animal characteristics (pathogen free, age, sex, strain, species) were reported and animals were from a commercial source (Charles River Japan)
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Not Rated	NA	NA	animal husbandry conditions were cited to another publication without further details
	Metric 15:	Number per Group	High	× 1	1	number of animals per group was reported (6 treated and 5 control) and adequate for study type
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	outcome assessment was cited to another publication and partially described and appeared appropriate for the outcome of interest

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Study Citation: Y. Higami, T. Tsuchiya, K. To, T. Chiba, H. Yamaza, D. Shiokawa, S. Tanuma, I. Shimokawa (2004). Expression of DNase gamma during Fas-independent apoptotic DNA fragmentation in rodent hepatocytes Cell and Tissue Research, 316(3,3), 403-407

Data Type:

HERO ID: 194726

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was cited to another publication and briefly described; appeared consistent across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Results obtained by counting 200 microscopic fields (~20,000 hepatocytes each) per animal
	Metric 19:	Blinding of Assessors	Low	× 1	3	blinding was not reported. One investigator performed all assessments.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	negative control response for CCL4 experiment was not reported or noted in text
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 but unlikely to significantly impact DNA fragmentation results
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	statistical methods were reported and appropriate for the data
	Metric 24:	Reporting of Data	Low	× 2	6	Results for CCL4 group described qualitatively in text; results for negative controls not reported.
Overall Quality Determination‡			Unacceptable**		1.8	
Extracted			No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 82: **Animal toxicity evaluation results of Barbin et al., 1983 study on DNA damage in rat liver**

Study Citation:	A. Barbin, J. C. Béréziat, H. Bartsch (1983). Evaluation of DNA damage by the alkaline elution technique in liver, kidneys and lungs of rats and hamsters treated with N-nitrosodialkylamines Carcinogenesis, 4(5,5), 541-545					
Data Type:	DNA damage rat liver					
HERO ID:	194728					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substance was identified by name
	Metric 2:	Test Substance Source	High	× 1	1	test substance source was reported (Prolabo, france), batch/lot was not reported
	Metric 3:	Test Substance Purity	Low	× 1	3	purity of the test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	concurrent negative untreated control animals were reported; the controls were not sham-treated but this is not likely to impact DNA damage findings
	Metric 5:	Positive Controls	Not Rated	NA	NA	not applicable for the study type
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals was not described
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage of the test substance were not reported. It appears that CCL4 was administered neat (vehicle was described for other chemicals tested). Given that the study was single exposure via i.p. , storage conditions are unlikely to impact the results.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration (e.g., time of day, volume of ip injection) were not reported but unlikely to substantially impact results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose was reported clearly (4 g/kg bw)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	single injection exposure reported and appropriate for the study
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose tested; dose selection was not justified and the only dose tested gave negative results
	Metric 12:	Exposure Route and Method	High	× 1	1	exposure route was appropriate for the substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	test animal characteristics (species, strain, sex, age, and initial bw) were reported. Animals were bred in house.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	animal husbandry conditions were not sufficiently reported (no information on cages, temperature, humidity).
	Metric 15:	Number per Group	Medium	× 1	2	number of animals per group was reported (4) and was slightly low, but sufficient for statistical analysis
Domain 5: Outcome Assessment						

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Study Citation:	A. Barbin, J. C. Béréziat, H. Bartsch (1983). Evaluation of DNA damage by the alkaline elution technique in liver, kidneys and lungs of rats and hamsters treated with N-nitrosodialkylamines Carcinogenesis, 4(5,5), 541-545
Data Type:	DNA damage rat liver
HERO ID:	194728

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	outcome assessment methodology was sensitive for the outcome of interest and described in detail.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcome assessment was consistent across study 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
	Metric 20:	Negative Control Response	High	× 1	1	negative controls response was reported and appeared to be adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences in initial body weight or food/water intake were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There was no attrition and no health outcomes unrelated to exposure were reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	statistical analysis was reported and appropriate for the data
	Metric 24:	Reporting of Data	High	× 2	2	data were reported for all outcomes and groups
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "Single dose level used without justification and it yielded negative results".

Table 83: **Animal toxicity evaluation results of Barrows et al., 1981 study on rat liver DNA methylation**

Study Citation:	L. R. Barrows,Shank RC (1981). Aberrant methylation of liver DNA in rats during hepatotoxicity Toxicology and Applied Pharmacology, 60(2,2), 334-345					
Data Type:	Rat liver DNA methylation					
HERO ID:	194757					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	tests substance was identified by name
	Metric 2:	Test Substance Source	High	× 1	1	test substance source was reported, Mallinckrodt, St Louis, MO
	Metric 3:	Test Substance Purity	High	× 1	1	test substance purity was reported as spectral grade, not further specified
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	concurrent negative controls were reported, however CCl4 administered in corn oil and controls received water
	Metric 5:	Positive Controls	Not Rated	NA	NA	not applicable for the study
	Metric 6:	Randomized Allocation	Low	× 1	3	method for allocation of animals was not described
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared in corn oil vehicle. Storage was not described but is unlikely to impact results given short duration of study
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	exposure administration was consistent across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose was reported clearly (1 g/kg bw)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	exposure frequency and duration (single dose) were reported and appropriate for the outcome of interest
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Single dose group; dose was not justified by the authors; however, the dose was sufficient to induce the outcome of interest
	Metric 12:	Exposure Route and Method	High	× 1	1	exposure route (oral gavage) was appropriate for the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Some test animal characteristics were reported (species, strain, sex, and body weight); animals were obtained from a commercial source (Charles River). No informatio non age or initial health provided.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Animal husbandry conditions were not sufficiently reported, but unlikely to substantially impact results given short duration of study
	Metric 15:	Number per Group	High	× 1	1	animal number per group was 4 treated, 5 control and was adequate for the study and analysis
Domain 5: Outcome Assessment						

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Study Citation:	L. R. Barrows,Shank RC (1981). Aberrant methylation of liver DNA in rats during hepatotoxicity Toxicology and Applied Pharmacology, 60(2,2), 334-345
Data Type:	Rat liver DNA methylation
HERO ID:	194757

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	the outcome assessment methodology addressed the outcome of interest, and was described in some detail with reference to published methods
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	outcome assessment was described. Table indicates that the amount of DNA analyzed for methylated guanine was much lower in the treated animals (4 mg) compared with controls (10 mg)
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Livers from 2 animals/group pooled for DNA extraction.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable to the study type
	Metric 20:	Negative Control Response	Medium	× 1	2	negative control responses were reported and appeared adequate (not detected) but limits of detection were not reported
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight was reported; food and water intake were not. However, given the brief duration of the study (single dose with sacrifice 12 hr later the lack of reporting is not likely to significantly impact results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	no health outcomes unrelated to exposures were reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	not applicable for the study type
	Metric 24:	Reporting of Data	Medium	× 2	4	data were from pooled sample (livers from 2 animals/group) and were reported for all outcomes
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 84: **Animal toxicity evaluation results of Ikegwuonu et al., 1991 study on DNA damage and repair in rat liver**

Study Citation:	F. I. Ikegwuonu, H. M. Mehendale (1991). Biochemical assessment of the genotoxicity of the in vitro interaction between chlordecone and carbon tetrachloride in rat hepatocytes Journal of Applied Toxicology, 11(4,4), 303-310					
Data Type:	DNA damage and repair rat liver					
HERO ID:	194760					
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 molecular formula
	Metric 2:	Test Substance Source	High	× 1	1	test substance source was reported; Fisher Chem Co.
	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 vehicle (corn oil) controls were reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	no positive control was used, but treatment-related positive responses were observed
	Metric 6:	Randomized Allocation	Low	× 1	3	method of allocation of animals was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	test material was inferred to be prepared in corn oil, storage was not reported but unlikely to impact results given short duration of study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	exposure administration was consistent across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose was reported as 100 uL CCL4/kg bw
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure, appropriate for study outcome.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Single dose was used; dose level was not justified by authors, but was sufficient to induce expected effect (UDS).
	Metric 12:	Exposure Route and Method	Low	× 1	3	i.p. administration is not recommended for UDS determination in hepatocytes because liver is exposed directly
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were partially reported (species, strain, sex, and body weight at study initiation reported; health status and age were not reported). Animals obtained from a commercial source (Charles River).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	animal husbandry conditions were reported (housing, light/dark cycle, temperature and RH) and adequate
	Metric 15:	Number per Group	High	× 1	1	animals per group was reported (3) and was adequate for statistical analysis
Domain 5: Outcome Assessment						

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Study Citation:	F. I. Ikegwuonu, H. M. Mehendale (1991). Biochemical assessment of the genotoxicity of the in vitro interaction between chlordecone and carbon tetrachloride in rat hepatocytes Journal of Applied Toxicology, 11(4,4), 303-310
Data Type:	DNA damage and repair rat liver
HERO ID:	194760

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	outcome assessment methodology was described in detail with some reference to published methods. Outcome assessment methods were appropriate for the outcome of interest. The duration between treatment and sacrifice was brief (1 hr) relative to recommendations (2-4 or 12-16 hr) but a clear positive response was seen.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcome assessment protocol was reported and assessment carried out consistently across all study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	5 ml hepatocyte suspension containing 1 x 10ˆ6 cells/ml, in triplicate
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study type
	Metric 20:	Negative Control Response	High	× 1	1	negative control responses were reported and appropriate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions (bw, food or water intake) of animals in each study group not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	data on health outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and adequate for the data set
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes including means, SEs, and number determinations
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 85: **Animal toxicity evaluation results of Iwai et al., 2002 study on oxidative DNA damage in rat liver**

Study Citation:	S. Iwai, R. Karim, M. Kitano, T. Sukata, W. Min, K. Morimura, H. Wanibuchi, S. Seki, S. Fukushima (2002). Role of oxidative DNA damage caused by carbon tetrachloride-induced liver injury -- enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats Cancer Letters, 179(1,1), 15-24
Data Type:	Oxidative DNA damage in rat liver
HERO ID:	194769

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substance was identified by name
	Metric 2:	Test Substance Source	High	× 1	1	test substance source was reported (Wako Pure Chemical Industries,Osaka, Japan).
	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 vehicle controls were reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	not applicable for the study type
	Metric 6:	Randomized Allocation	Low	× 1	3	method of animal allocation was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in a corn oil vehicle but it is unclear if prepared fresh for each dose. Storage was not reported
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	exposure administration (e.g., sc injection volume) was consistent across groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose was reported in ml/kg body weight (0.25) and can be converted to mg/kg/day
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	exposure frequency (2x week) and duration (1 wk) were reported and and adequate for the study
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	One dose group was used. The dose was not justified by authors. A positive response was observed with the one dose, indicating that it was high enough to elicit a response (oxidative DNA damage).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	exposure route and method of exposure were reported, but location of s.c. administration was not. Route and method were acceptable for the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Reported test animal characteristics include species, strain, sex, and age; body weight and health condition were not reported. Animals obtained from a commercial source (Charles River Japan)
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions reported (temperature, RH, and light cycle; housing conditions were not reported), adequate, and similar across groups.

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Study Citation:	S. Iwai, R. Karim, M. Kitano, T. Sukata, W. Min, K. Morimura, H. Wanibuchi, S. Seki, S. Fukushima (2002). Role of oxidative DNA damage caused by carbon tetrachloride-induced liver injury -- enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats Cancer Letters, 179(1,1), 15-24
Data Type:	Oxidative DNA damage in rat liver
HERO ID:	194769

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	Number of animals per group was reported (5) and was adequate for the outcome analysis
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was briefly described and cited to published method. The methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was described and consistent across study 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
	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	Medium	× 2	4	Initial body weights and food and water intake were not reported but these are unlikely to significantly impact the results
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on health outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	statistical methods were reported and adequate for the data set
	Metric 24:	Reporting of Data	High	× 2	2	8-OHdG levels in rat liver were reported graphically with error bars for all groups
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 86: **Animal toxicity evaluation results of Bermudez et al., 1982 study on in vivo DNA damage in heptocytes from treated rats**

Study Citation:	E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679
Data Type:	in vivo DNA damage in heptocytes from treated rats
HERO ID:	194786

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Fisher Scientific Co). 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	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative solvent (corn oil) controls were used
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used; however, treatment-related positive responses were observed for other chemicals tested in the study demonstrating that the test is capable of detecting a positive response.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was reported to have been prepared in corn oil. The storage of the test substance was not reported; however, it was a single administration so storage is unlikely to affect results.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported, but with limited details. Gavage volume was not excessive (0.4 mL/100 g bw). Animals were treated with a single exposure, so these limitations are unlikely to have a substantial impact on results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered dose was reported without ambiguity (400 mg/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint; single oral dose
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose was tested. The administered dose level was not justified, and results were all negative; it is not certain if a higher dose would elicit a response for this outcome.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance (gavage, in corn oil)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and weight were reported. The commercial source was reported (Charles River Breeding labs). The health status and age were not reported. The test species and strain were an appropriate animal model for the evaluation of this endpoint

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Study Citation:	E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679
Data Type:	in vivo DNA damage in heptocytes from treated rats
HERO ID:	194786

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported (only feed type reported) to evaluate if husbandry was adequate.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (2 animals/group) was lower than the typical number used in similar types of studies
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline elution) was reported and appropriate for the endpoint 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	Sampling was adequate for the outcomes of interest (cell concentration: 1x10+6/ml)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study type
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and 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	Not Rated	NA	NA	Statistical analysis between treated and control groups was not conducted. The results indicate if a treatment is considered negative or positive. Statistical analysis may not be necessary for this study. There were negative findings for CCl4-treated groups at all time points.
	Metric 24:	Reporting of Data	Low	× 2	6	DNA damage results were presented quantitatively for all time points for treated animal. Results for solvent controls were pooled across both water controls and corn oil controls (appropriate for CCL4 experiment) and time points. It is unclear whether the quantitative results for alkaline elution, given in % of control, were based on pooled or individual control group results.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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Study Citation:	E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679
Data Type:	in vivo DNA damage in heptocytes from treated rats
HERO ID:	194786

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 87: **Animal toxicity evaluation results of Kadiiska et al., 2005 study on DNA adducts**

Study Citation:	M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710
Data Type:	DNA adducts for CCl4
HERO ID:	194788

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance source (a manufacturer) was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition.
	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 negative (vehicle-only) controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type (the detection of adducts is indicative of a positive result).
	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 was inferred to be dissolved in vehicle. "Sample preparation" was cited to another publication. Storage was not reported, but not likely to impact the study results (owing to the short-term nature of the study).
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Animal treatment was cited to other publications.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported clearly.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration (i.e., single gavage dose) were reported and adequate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups (2 doses plus controls) was reported and considered adequate to address the purpose of the study. Although doses were justified based on previous studies, toxicity (evidenced by serum enzyme activities) was evident at both doses.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route (intraperitoneal injection) is suited to the test substance but not environmentally relevant.
Domain 4: Test Organism						

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Study Citation:	M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710
Data Type:	DNA adducts for CCl4
HERO ID:	194788

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were partially reported (obtained from a commercial source, sex, strain, range of body weights). Minor uncertainties in the reporting of test animal characteristics (e.g., health status and age) are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions (temperature, humidity, light/dark) were reported and appropriate for the study.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (5 males) was reported and adequate for analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcome of interest. and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was reported. The study indicated that samples were marked with a code number so that those conducting assays were unaware of treatment status.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded appropriately (low levels of adducts).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate for the data set.
	Metric 24:	Reporting of Data	High	× 2	2	Data was reported for all groups/time points.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710
Data Type:	DNA adducts for CCl4
HERO ID:	194788

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 88: **Animal toxicity evaluation results of Kadiiska et al., 2005 study on DNA damage**

Study Citation:	M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710
Data Type:	DNA damage for CCl4
HERO ID:	194788

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance source (a manufacturer) was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition.
	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 negative (vehicle-only) controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable 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 was inferred to be dissolved in vehicle. "Sample preparation" was cited to another publication. Storage was not reported, but not likely to impact the study results (owing to the short-term nature of the study).
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Animal treatment was cited to other publications.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported clearly.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration (i.e., single gavage dose) were reported and adequate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (2 doses plus controls) was reported and considered adequate to address the purpose of the study. Although doses were justified based on previous studies, toxicity (evidenced by serum enzyme activities) was evident at both doses.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route (intraperitoneal injection) is suited to the test substance but not environmentally relevant.
Domain 4: Test Organism						

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Study Citation: M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710

Data Type: DNA damage for CCl4

HERO ID: 194788

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were partially reported (obtained from a commercial source, sex, strain, range of body weights). Minor uncertainties in the reporting of test animal characteristics (e.g., health status and age) are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions (temperature, humidity, light/dark) were reported and appropriate for the study.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (5 males) was reported and adequate for analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was cited to another publication; little information was provided in the study. It is not clear that methods were sensitive for the outcome since effects were only observed at the lowest tested dose.
	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 for the study type.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was reported. The study indicated that samples were marked with a code number so that those conducting assays were unaware of treatment status.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	The biological responses of the negative control groups were not reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate for the data set.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Data presentation was inadequate. Although study results (and their statistical significance) were discussed in the text (roughly by exposure group and time point), data were not quantified; the information reported was not sufficient for an independent interpretation of the study results (particularly the positive result seen only at the low dose at one time point).
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.0	

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Study Citation:	M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plastaras,Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710
Data Type:	DNA damage for CCl4
HERO ID:	194788

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

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 89: **Animal toxicity evaluation results of Diaz Gomez et al., 1980 study on binding to rat and mouse liver DNA**

Study Citation:	M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206
Data Type:	Binding to rat and mouse liver DNA for CCl4
HERO ID:	194790

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	Manufacturer of radiolabeled test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	It was indicated that the test substance was 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls were not needed for the primary DNA binding experiment. Vehicle controls were used for some supplemental experiments evaluating changes in response to a tracer dose of radiolabeled CCl4 (Table 2).
	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	Medium	× 1	2	Preparation in olive oil was described; storage was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure appeared consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Specific activity was provided for radiolabeled substances. The toxic dose of CCl4 could be calculated from the information provided.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration were reported (single injection dose).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The use of a single dose is adequate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Intraperitoneal injection in olive oil is appropriate for the test substance, but this is not an environmentally relevant route.
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 sufficiently reported.
	Metric 15:	Number per Group	Medium	× 1	2	3 rats/group; 30 mice/group (livers from 10 mice were pooled for each sample)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methods were partially described and cited to several other publications.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.

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Study Citation:	M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206
Data Type:	Binding to rat and mouse liver DNA for CCl4
HERO ID:	194790

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls were not necessary for the primary experiment.
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 treatment group.
	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	The statistical methods were described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented for all outcomes.
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 90: **Animal toxicity evaluation results of Draper et al., 1995 study on deoxyguanine-MDA adducts**

Study Citation:	H. H. Draper, S. Agarwal, D. E. Nelson, J. J. Wee, A. K. Ghoshal, E. Farber (1995). Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA Lipids, 30(10,10), 959-961
Data Type:	Deoxyguanine-MDA adducts for CCl4
HERO ID:	194814

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	Low	× 1	3	Source was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	In Table 1, the study authors indicated the use of a concurrent negative control group, but details regarding the negative control group were not reported.
	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	CCl4 was apparently administered neat by oral gavage (10 uL).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Dose can be calculated from the gavage volume assuming 100 g bw (approximate weight specified in the methods for the CCl4 experiment).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose is adequate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose level was not justified.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Oral gavage is an appropriate route, but a vehicle should be used to avoid damage to the gastrointestinal tract (unclear that a vehicle was used).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source, strain, and sex of test animals 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	The number of animals used was reported (5/group) and is adequate for the outcome of interest.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	The outcome assessment methods were cited to Agarwal and Draper (1992).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	The outcome assessment methods were cited to Agarwal and Draper (1992).

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Study Citation:	H. H. Draper, S. Agarwal, D. E. Nelson, J. J. Wee, A. K. Ghoshal, E. Farber (1995). Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA Lipids, 30(10,10), 959-961
Data Type:	Deoxyguanine-MDA adducts for CCl4
HERO ID:	194814

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric does not apply to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric does not apply to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response appears adequate.
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 across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistics were performed, but the method was not reported.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported as mean +/-SEM.
Overall Quality Determination[‡]			Low		2.4	
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 91: **Animal toxicity evaluation results of Rocchi et al., 1973 study on DNA binding**

Study Citation:	P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425
Data Type:	DNA binding for CCl4
HERO ID:	194878

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 radiolabeled carbon tetrachloride (CCl4-14C).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the radiolabeled test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The specific activity of the CCl4-14C was reported. The test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative control groups were not included (and not necessarily required by the study type). It was noted that the counts per minute (cpm) of samples were at least twice background levels. However, controls were not mentioned, and it was not indicated how samples were corrected for radioactivity levels.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. The identification of labeled DNA was evidence of a positive result. Methylcholanthracene pre-treatment was used to stimulate hepatic metabolism.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details regarding preparation of the test substance were limited (dissolved in corn oil). Storage of the test substance was 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 appropriate and consistent among study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The dose used in the study was reported (326 UCi corresponding to 367 umol/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency/duration (single injection) were reported and appropriate for the study design.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Only one dose level was utilized, but this is considered to be adequate given the study design. A rationale for the selection of this dose level was not provided.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route of exposure was reported and appropriate (but not environmentally relevant).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animals were not reported. The species, strain, life stage (adult), sex, and point estimate of body weight were reported.

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Study Citation:	P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425
Data Type:	DNA binding for CCl4
HERO ID:	194878

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.
	Metric 15:	Number per Group	Low	× 1	3	There were 3 rats per treatment group and 25 mice per treatment group; however livers were pooled by treatment group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was partially described (e.g., radioactivity measurement) and partially cited to other sources (isolation of DNA, RNA, and protein fractions).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently among treatment 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.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This metric is not applicable to the study design, as no negative controls were utilized.
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	No confounding variables unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Since livers were pooled by treatment group, no standard deviations were reported, and statistical analysis was not possible.
	Metric 24:	Reporting of Data	Low	× 2	6	Data were reported by group (pre-treated or not; without control data or a measure of variation).
Overall Quality Determination‡			Unacceptable**		2.2	
Extracted			No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 92: **Animal toxicity evaluation results of Gans et al., 1984 study on DNA damage**

Study Citation:	J. H. Gans, R. Korson (1984). Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy Proceedings of the Society for Experimental Biology and Medicine, 175(2,2), 237-242					
Data Type:	DNA damage for CCl4					
HERO ID:	194904					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Fisher Scientific Co). 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	Medium	× 1	2	The purity of the test substance reported to be technical grade; impurities are not likely to impact the study results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative (vehicle-only) controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used; however, treatment-related positive responses were observed in the study demonstrating that the test is capable of detecting a positive response.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was reported to have been prepared in corn oil. The storage of the test substance was not reported; however, it was a single dose adminsitration.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and appeared consistent.
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The range of administered doses was reported (0.02 – 0.10 µl or 0.134-0.67 µmol/g body weight). Two (but apparently not all) of the doses (in uL/g body weight) were reported in Figure 1. It is unclear how the body weight of mice could be estimated (age when received at 20 to 25 g of weight not reported; DNA damage assay performed in animals that were 4 months of age).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure/frequency were reported (single dose) and appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	The number of exposure groups was not reported (at least 2 dose groups plus controls based on data presented in Figure 1); administered doses were reported as a range. Doses were presumably selected based on previous studies (not explicitly specified).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance (gavage).
Domain 4: Test Organism						

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Study Citation:	J. H. Gans, R. Korson (1984). Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy Proceedings of the Society for Experimental Biology and Medicine, 175(2,2), 237-242
Data Type:	DNA damage for CCl4
HERO ID:	194904

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and weight (range) were reported. The commercial source was reported (Charles River); health status and age were not reported. The test species and strain were an appropriate animal model for the evaluation of this endpoint.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were partially described with some omissions (temperature and humidity).
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per group was not reported; it was noted that there were 9 mice in the control group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodologies were partially described; the methodology was further described in previously published papers (Cox et al., 1973 and Laishes et al., 1975)
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment methodologies were described in previously published papers (Cox et al., 1973 and Laishes et al., 1975), but some details were briefly described and appeared consistent.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for this study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study type.
	Metric 20:	Negative Control Response	Low	× 1	3	The control response was reported; the responses appear adequate; however, there is some uncertainty in the reporting (only limits of sedimentation were reported graphically).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions for each study group is 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	Unacceptable	× 1	4	Statistical analysis between treated and control groups was not conducted for the alkaline elution study. Data are not amenable to independent analysis.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group; data were only reported for some groups/outcomes.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.3	
Extracted			No			

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Study Citation:	J. H. Gans, R. Korson (1984). Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy Proceedings of the Society for Experimental Biology and Medicine, 175(2,2), 237-242
Data Type:	DNA damage for CCl4
HERO ID:	194904

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 93: **Animal toxicity evaluation results of Sarkar et al., 1999 study on chromosomal aberrations**

Study Citation:	A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by Trianthema portulacastrum L. in carbon tetrachloride-induced mouse liver damage Cell Biology International, 23(10,10), 703-708
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194915

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 carbon tetrachloride (CCl4).
	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, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The grade/purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (vehicle-only) control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not used; however, positive responses were observed in this study (i.e., demonstrating the test is capable of detecting a positive response).
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that animals were randomly allocated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	It was indicated that the test substance was dissolved in olive oil; however, storage was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volumes were not consistently reported. Based on the information provided, it appears that exposures were applied consistently (7 weeks daily distilled water plus vehicle-only or CCl4 exposure three times weekly for 5 weeks).
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The dose was reported as 20%/mouse; the dose in mg/kg/day cannot be determined.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Frequency/duration of exposure was reported (3 times/week for 5 weeks). The applicability of a repeated-dose protocol to this study type is uncertain.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The study utilized one dose of CCl4; the abstract indicated that the dose was necrogenic. Although no rationale was provided, the study authors indicated that the dose used was adequate to increase the percentage of aberrant metaphase cells.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route/method was reported and appropriate (i.e., oral gavage).
Domain 4: Test Organism						

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Study Citation:	A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by Trianthema portulacastrum L. in carbon tetrachloride-induced mouse liver damage Cell Biology International, 23(10,10), 703-708
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194915

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Minor uncertainties in the reporting of test animal characteristics (e.g., health status, age) are unlikely to have a substantial impact on results. The test animals were obtained from a commercial source.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported (temperature, humidity, light-dark cycle).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that there were 15-22 mice/group; based on Table 1 (chromosomal aberrations), data were for 5-7 mice/time point (15, 30, or 45 days after exposure).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was partially described and partially cited to other publications (e.g., chromosome preparation cited to Horiuchi et al., 1984). In addition, it is not clear that the reported outcome assessment methodology was appropriate for the outcome of interest (e.g., evaluation of major structural chromosomal aberrations 15, 30, and 45 days after the last CCl4 dose).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Based on data in Table 1, major chromosomal aberrations were scored in each group at the same time points after treatment.
	Metric 18:	Sampling Adequacy	Low	× 1	3	The study indicated that 50 metaphases were analyzed by animal (or about 250/group); this is fewer than recommended for studies of this type.
	Metric 19:	Blinding of Assessors	High	× 1	1	The study indicated that, for chromosomal aberrations, coded slides were scored blind.
	Metric 20:	Negative Control Response	High	× 1	1	The study authors indicated that the response of the negative control group was adequate (e.g., low numbers of chromosomal aberrations).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Information regarding initial animal conditions were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	The study indicated that "data were analyzed statistically for differences between the mean using Student's t-test" and that significance was established at $p < 0.05$. However, it does not appear that comparisons were made between controls and CCl4-treated groups, and data are not provided (by time point) for independent analyses.

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Study Citation:	A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by Trianthema portulacastrum L. in carbon tetrachloride-induced mouse liver damage Cell Biology International, 23(10,10), 703-708
Data Type:	Chromosomal aberrations for CCl4
HERO ID:	194915

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 24: Reporting of Data	Unacceptable	× 2	8	Data for CCl4 treatment groups are provided by time point; however, the table indicates only the mean value for the control group (overall).
Overall Quality Determination[‡]		Unacceptable[⋆⋆]		2.2	
Extracted		No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 94: **Animal toxicity evaluation results of Sawada et al., 1991 study on rat liver chromosome aberrtion, micronuclei, SCE**

Study Citation:	S. Sawada, T. Yamanaka, K. Yamatsu, C. Furihata, T. Matsushima (1991). Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced in vivo by hepatocarcinogens including heterocyclic amines Mutation Research, 251(1,1), 59-69
Data Type:	Rat liver chromosome aberrtion, micronuclei, SCE
HERO ID:	194926

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, formula and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Source of the test substance was Wako Pure Industries, Osaka.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle control group was used for a negative control.
	Metric 5:	Positive Controls	High	× 1	1	Positive responses were elicited with other substances tested including DMN and 2-AAF.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in corn oil; however, storage conditions or solution are not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistent and appropriate across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported without ambiguity (1600mg/kg).
	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	Low	× 1	3	Only one concentration of test substance was studied, which produced negative results. It is unclear if a higher dose would have elicited positive responses.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Information on test animals was provided, purchased from Charles River Japan, Kanagawa.
	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	3-4 rats per time point were studied.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methodology is partially described and cited elsewhere.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Minor details regarding outcome assessment protocol are not provided, this is unlikely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not applicable to this study.

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Study Citation:	S. Sawada, T. Yamanaka, K. Yamatsu, C. Furihata, T. Matsushima (1991). Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced in vivo by hepatocarcinogens including heterocyclic amines Mutation Research, 251(1,1), 59-69
Data Type:	Rat liver chromosome aberrtion, micronuclei, SCE
HERO ID:	194926

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	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	Low	× 2	6	Initial conditions of test animals were not fully reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on health status after treatment are not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis performed was appropriate (t-test).
	Metric 24:	Reporting of Data	High	× 2	2	Data for all outcomes were reported.
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 95: **Animal toxicity evaluation results of Brambilla et al., 1983 study on fragmentation of liver DNA**

Study Citation:	G. Brambilla, P. Carlo, R. Finollo, F. A. Bignone, A. Ledda, E. Cajelli (1983). Viscometric detection of liver DNA fragmentation in rats treated with minimal doses of chemical carcinogens Cancer Research, 43(1,1), 202-209					
Data Type:	Fragmentation of liver DNA					
HERO ID:	194933					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was E. Merck (Darmstadt, West Germany)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls rats were administered the vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not employed, however positive responses were elicited with other test substances.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on how test substance was prepared was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported as 200 mg/kg.
	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	Low	× 1	3	Only one dose was studied which resulted in a negative response. It is unclear if higher doses would have elicited a response.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Source of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Three rats / groups were studied.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was partially described and cited elsewhere.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were incomplete reporting of outcome assessment methodology.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable to this study.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was adequate.

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Study Citation:	G. Brambilla, P. Carlo, R. Finollo, F. A. Bignone, A. Ledda, E. Cajelli (1983). Viscometric detection of liver DNA fragmentation in rats treated with minimal doses of chemical carcinogens Cancer Research, 43(1,1), 202-209
Data Type:	Fragmentation of liver DNA
HERO ID:	194933

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions or animals were not fully reported.
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	Health conditions of animals after treatment were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical analysis was performed and appropriate.
	Metric 24: Reporting of Data	High	× 2	2	Data were fully reported in Table 1.
Overall Quality Determination‡		Unacceptable**		1.9	
Extracted		No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 96: **Animal toxicity evaluation results of Levy et al., 1984 study on mitochondrial and nuclear DNA binding in rat liver**

Study Citation:	G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234
Data Type:	mitochondrial and nuclear DNA binding in rat liver
HERO ID:	194952

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Amersham). 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 of the test substance was reported (>99%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	The use of a negative control group is not necessary for DNA binding assays; measurement of radiolabeled test compound is the outcome.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used; however, is not necessary for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported. The storage of the test substance was not reported; however, it was a single administration (unlikely to affect results).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and appear to be administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered dose was reported. (2 µl/kg = 2.1 x 10-2 µmol/kg; 1.1 ml/kg = 11.4 µmol/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint; single dose
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 doses were tested and 2 time points for the low dose group; known non-necrotizing or acutely toxic doses.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The route or method of exposure was not reported; it could be oral or some injection route, but the study does not specify.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and weight were reported. Health status and age were not reported. The commercial source was reported (Charles River). The test species and strain were an appropriate animal model for the evaluation of this endpoint.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per study group was not reported

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Study Citation:	G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234
Data Type:	mitochondrial and nuclear DNA binding in rat liver
HERO ID:	194952

Domain	Metric		Rating†	MWF⋆	Score	Comments††
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	Medium	× 1	2	Details of the outcome assessment protocol were reported; 2 time points were analyzed for the low dose group (5 and 24 hours), but only analyzed at 1 time point (24 hours) for the high dose group.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Details of sampling of outcomes were not clearly reported; number of animals tested were not reported; DNA binding results were calculated based on 3.237 umol/mg DNA
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study type
	Metric 20:	Negative Control Response	Not Rated	NA	NA	The use of a negative control group is not necessary for DNA binding assays
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight was reported, though 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	Medium	× 1	2	Statistical analysis was not conducted. DNA binding data were provided as a mean and standard deviations for a specified number of experiments; independent statistical analysis may be performed.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for dose groups, preparations, and time points with quantal presentation.
Overall Quality Determination‡			Unacceptable**		1.8	
Extracted			No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 97: **Animal toxicity evaluation results of Cabra et al., 1999 study on DNA damage**

Study Citation:	M. Cabré, N. Ferré, J. Folch, J. L. Paternain, M. Hernàndez, D. Del Castillo, J. Joven, J. Camps (1999). Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats Journal of Hepatology, 31(2,2), 228-234
Data Type:	DNA damage for CCl4
HERO ID:	194968

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 carbon tetrachloride.
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance source 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	High	× 2	2	Concurrent negative controls received vehicle injection (olive oil).
	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	Medium	× 1	2	Preparation of the test substance (1:1 dilution in olive oil) was reported. Storage of the test substance between injections was not reported. This deficiency is not expected to have substantially impacted results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The dose of CCl4 was described as 0.5 mL CCl4 per kg body weight. This was calculated by the reviewer to be 795 mg/kg based on the density of CCl4 (1.59 g/mL).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency (two i.p. injections 4 days apart) and duration (sacrificed 1 day after second injection) was considered to be appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Only one dose level was utilized. The dose level was considered to be adequate, as positive results were observed.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was 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. This is not expected to have substantially impacted results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	It was described that animals were on a reverse 12-hour light cycle. The justification for this reverse light cycle was not included. The temperature and humidity of the animal rooms were not reported. These deficiencies are not expected to have substantially impacted results.

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Study Citation:	M. Cabré, N. Ferré, J. Folch, J. L. Paternain, M. Hernàndez, D. Del Castillo, J. Joven, J. Camps (1999). Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats Journal of Hepatology, 31(2,2), 228-234
Data Type:	DNA damage for CCl4
HERO ID:	194968

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The control group had n = 11 rats and the CCl4-only group had n = 12 rats. These numbers are considered adequate for the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate to address the intended outcome (DNA fragmentation). The TUNEL assay is usually utilized to assess DNA fragmentation as a result of apoptosis, but DNA strand breaks from other causes will be detected as well by this assay.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	It was noted that 25,000-30,000 cells per slide were counted. It was not clear how many slides per animal were included.
	Metric 19:	Blinding of Assessors	High	× 1	1	It was noted that the identity of the slides was coded prior to analysis.
	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	Information on initial conditions for each study group is not reported (body weight, food, water intake).
	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	Data were analyzed appropriately.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported adequately.
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 98: **Animal toxicity evaluation results of Castro et al., 1989 study on DNA and nuclear protein binding in vivo**

Study Citation:	G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294
Data Type:	DNA and nuclear protein binding in vivo
HERO ID:	194983

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as CCL4, CASRN provided. Radiolabeled CCL4 also used.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial sources were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Unlabeled CCL4 reported to be "low sulfur quality", - labeled CCL4 purity 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Not applicable for the study design
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for the study design
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Not applicable, only a single group of animals were used and random allocation is not necessary.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Storage of radiolabeled CCL4 was reported and stability was tested/confirmed. Test substance was prepared as a 15% v/v/ solution in olive oil prior to administration.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure were reported, and volume was appropriate (5ml/kg, ip)
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose was not reported in mg/kg bw, but as 1.1 x 10ˆ8 d.p.m/mL solution administered at a dose of 5 mL/kg. Animal body weight ranges were provided. Justification for the chosen dose was not provided.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure and duration were appropriate for the outcome of interest. Single injection, animals were sacrificed after 6 hours
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single exposure group was acceptable and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route (i.p) was acceptable for the test substance and for the outcome of interest.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Three species (rat, mouse, hamster) were included. Strains, sex, and body weight ranges were provided. The sources of the animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.

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Study Citation:	G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294
Data Type:	DNA and nuclear protein binding in vivo
HERO ID:	194983

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15: Number per Group	High	× 1	1	The number of animals per group was appropriate. Triplicate experiments were done on pooled samples from 6 mice, 2 hamsters, and 1 rat per sample.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was appropriate for the outcome of interest. Some details were described, some methods were cited to another publication.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Consistency in outcome assessment between replicates was assumed from the text.
	Metric 18: Sampling Adequacy	High	× 1	1	Sampling adequacy was appropriate for the outcome of interest (sufficient concentrations of covalently bound DNA and protein to accurately detect above background)
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Not applicable for the outcome of interest.
	Metric 20: Negative Control Response	Not Rated	NA	NA	Not applicable (no negative control)
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Confounding variables in the test design and procedure were not reported. Since only one group of animals were tested, there were no concerns about potential differences between groups
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes unrelated to exposure (e.g., animal health prior to the start of the study) were not reported. Since only one group of animals were tested, there were no concerns about potential differences between groups
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical analysis to evaluate differences between species was performed and appropriate (student T-test). Data were presented as Means with SD
	Metric 24: Reporting of Data	High	× 2	2	Data for all of the outcomes of interest were adequately 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.

Table 99: **Animal toxicity evaluation results of Craddock et al., 1978 study on unscheduled DNA synthesis in rat liver after i.p. exposure**

Study Citation:	V. M. Craddock, A. R. Henderson (1978). De novo and repair replication of DNA in liver of carcinogen-treated animals Cancer Research, 38(7,7), 2135-2143					
Data Type:	Unscheduled DNA synthesis in rat liver after i.p. exposure					
HERO ID:	195014					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was Fisons Scientific Apparatus Ltd. (Loughborough, Leicestershire, England.)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control was not used. Untreated rat data came from a previously published study (Craddock, 1976).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control was not employed, however positive responses were elicited with other test substances.
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was mixed with equal volume of liquid paraffin, however concentration of prepared mixture (mg/ml) was not reported nor were storage conditions. Storage conditions unlikely to substantially impact results of this single exposure study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently, and gavage volume was appropriate (reported as 2.5 ml/kg in Chart 4 ; CCL4 was prepared in an equal volume of liquid paraffin yielding gavage volume of 5 ml/kg)
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose is reported as 2.5 ml/kg in Chart 4 or 4000 mg/kg in Table 1 (doses equivalent based on density of 1.59 g/ml).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/ duration (once) were reported and appropriate for this study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Dose used was justified as previously shown to cause necrosis at later timepoints. In the absence of a negative control it is not possible to determine whether the dose was appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route (gavage) was appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The species, strain, sec, and initial body weight of test animals were reported. The source of test animals 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:	V. M. Craddock, A. R. Henderson (1978). De novo and repair replication of DNA in liver of carcinogen-treated animals Cancer Research, 38(7,7), 2135-2143
Data Type:	Unscheduled DNA synthesis in rat liver after i.p. exposure
HERO ID:	195014

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	4 animals/groups were studied. This is lower than the typical number (5/group) for this type of study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methodology is partially described and cited elsewhere.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of outcome assessment protocol; however limitations are unlikely to have substantial impact on results.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for outcome of interest (4 g liver from each animal pooled for nuclei isolation)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not applicable to this study.
	Metric 20:	Negative Control Response	Low	× 1	3	The biological response of negative control group was adequate; however, data were obtained from previous study (Craddock, 1976) rather than concurrent with this experiment
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions (food/water intake, health condition) of rats are not fully reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition or health outcomes unrelated to exposure are not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical analysis is not necessary for this outcome
	Metric 24:	Reporting of Data	High	× 2	2	Results for exposed groups are reported.
Overall Quality Determination‡			Unacceptable**		2.1	
Extracted			No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 100: **Animal toxicity evaluation results of Nakamura et al., 1992 study on DNA strand breaks for CCl4 (acute and chronic)**

Study Citation:	T. Nakamura, M. Hotchi (1992). Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury Virchows Archiv B Cell Pathology Including Molecular Pathology, 63(1,1), 11-16
Data Type:	DNA strand breaks for CCl4 (acute and chronic)
HERO ID:	195152

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 carbon tetrachloride (CCl4).
	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, 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	Low	× 2	6	Results section compares data to “untreated controls”. Details pertaining to the control are not provided. The study does indicate that for in situ nick translation (ISNT), sections treated without DNA polymerase (unstained) were used as a negative control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not employed (or required by study type).
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was provided (dissolved in olive oil); however, storage conditions were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across/within study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Dose concentrations were reported as 20% CCl4 administered at 5mL/kg (acute study) and 50% CCl4 administered at 2.5mL/kg ("chronic" study). Doses can be estimated based on (initial) approximate body weight provided (150 g); however, body weights would be expected to change over the course of the chronic study (12 weeks).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency/duration of exposures were reported (once for the "acute" study, and twice weekly for up to 12 weeks for the "chronic" study).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups was reported (1 dose group for each study, evaluated over multiple time points). A rationale for the selected doses was not provided (presumably based on previous studies).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route of exposure was reported (intraperitoneal or subcutaneous injection), but not environmentally relevant.
Domain 4: Test Organism						

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Study Citation:	T. Nakamura, M. Hotchi (1992). Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury Virchows Archiv B Cell Pathology Including Molecular Pathology, 63(1,1), 11-16
Data Type:	DNA strand breaks for CCl4 (acute and chronic)
HERO ID:	195152

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	High	× 2	2	Pertinent information on test animal was reported (source, strain, sex, age, initial body weights).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Based on information provided in the methods section with respect to the number of animals subjected to acute and chronic exposure and the time points of sacrifice, there were likely 3-4/rats/time point (fewer than recommended by study type).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology is described (original source of the method is also cited). It is noted that the methodology was applied to evaluate DNA strand breaks as a marker for proliferation, differentiation, and/or activated gene expression (rather than DNA injury).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It appears that outcomes were assessed consistently in the study groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details on sampling outcomes were limited. The legend to Figure 2 describes data as the number of positive cells/10 high power fields (presumably expressed as a mean for animals evaluated, but not specified).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 20:	Negative Control Response	Medium	× 1	2	Some data for untreated animals were described qualitatively on the text; data for the 0 hour/week time point were shown in Figure 2.
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	Health outcomes of study groups (unrelated to exposure) were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analyses were not described. The data presented in Figure 2 are not amenable to independent analyses because the "n" and measure of variance (SD or SEM) shown are not clearly reported.
	Metric 24:	Reporting of Data		× 2	NA	Data for each study (acute and chronic) by time point are presented in Figure 2.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		0.0	

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Study Citation:	T. Nakamura, M. Hotchi (1992). Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury Virchows Archiv B Cell Pathology Including Molecular Pathology, 63(1,1), 11-16
Data Type:	DNA strand breaks for CCl4 (acute and chronic)
HERO ID:	195152

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

[**] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 101: **Animal toxicity evaluation results of LÃ³pez-Diazguerrero et al., 2005 study on comparison of oxidative DNA damage in young and old mice**

Study Citation:	N. E. López-Diazguerrero, A. Luna-López, M. C. Gutiérrez-Ruiz, A. Zentella, M. Königsberg (2005). Susceptibility of DNA to oxidative stressors in young and aging mice Life Sciences, 77(22,22), 2840-2854
Data Type:	Comparison of oxidative DNA damage in young and old mice
HERO ID:	195160

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	The source of test substance was identified as Sigma (ST. Louis, MO)
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of the highest analytical grade available.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative control group was treated concurrently with vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Treatment related positive responses were elicited.
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals is not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance is described; however, storage conditions are not. As the study was only 3 days in duration, the lack of storage information is unlikely to significantly impact the results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Animals were exposed consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported as 0.16 ml/kg,
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency (once per day) and duration (3 days) were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Only one dose was used; it was not justified by the authors, but was sufficient to yield a positive response.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method (i.p. injection) were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, age, and source were reported. Test animals were obtained from a closed breeding colony from Universidad Autonoma Metropolitana-Iztapalapa.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were reported to be in accordance with Mexican official ethics standard; no further details provided
	Metric 15:	Number per Group	Medium	× 1	2	3 animals/group were used; this is a smaller size than typical for this outcome but sufficient for statistical analysis
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported fully and sensitive for the outcome of interest.

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Study Citation:	N. E. López-Diazguerrero, A. Luna-López, M. C. Gutiérrez-Ruiz, A. Zentella, M. Königsberg (2005). Susceptibility of DNA to oxidative stressors in young and aging mice Life Sciences, 77(22,22), 2840-2854
Data Type:	Comparison of oxidative DNA damage in young and old mice
HERO ID:	195160

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling is adequate for study design (100 ug samples DNA analyzed for 8-oxodGuo)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not necessary.
	Metric 20:	Negative Control Response	High	× 1	1	Response of negative controls was reported and appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial condition (body weight, food/water intake, health status) of animals is not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was described and appropriate (Student's t test.)
	Metric 24:	Reporting of Data	Medium	× 2	4	Data reported graphically for all groups. Legend of figure 1 states that data shown are mean ±SD of at least three independent experiments.
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 102: **Animal toxicity evaluation results of Kitta et al., 1982 study on DNA damage in rat liver after i.p. exposure**

Study Citation:	D. Kitta, M. Schwarz, H. A. Tennekes, H. Uehleke, W. Kunz (1982). Covalent binding of CCl4-intermediates to reduced pyridine nucleotides in mouse liver Advances in Experimental Medicine and Biology, 136 769-777
Data Type:	DNA damage in rat liver after i.p. exposure
HERO ID:	195226

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and molecular formula
	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	Low	× 2	6	Study authors reported concurrent negative control but it is not clear whether it was untreated or sham/vehicle treated
	Metric 5:	Positive Controls	Unacceptable	× 1	4	Positive control was not used and results generally negative.
	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	Unacceptable	× 1	4	Test substance preparation and storage were not reported. It is not clear whether a vehicle was used.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Available information was not sufficient to determine whether exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported clearly as 4 mg CCL4/kg bw
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose study, appropriate to outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose group included; dose was not justified by study authors, and results were negative
	Metric 12:	Exposure Route and Method	Low	× 1	3	i.p administration was used and was not justified by study authors. I.p. administration is generally not recommended for this study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Unacceptable	× 2	8	Strain, sex, age, body weight, and source of test animal were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate
	Metric 15:	Number per Group	Unacceptable	× 1	4	Number of animals per group was not reported
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methods cited to another publication with no additional details.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methods cited to another publication with no additional details.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding outcome sampling were not reported.

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Study Citation:	D. Kitta, M. Schwarz, H. A. Tennekes, H. Uehleke, W. Kunz (1982). Covalent binding of CCl4-intermediates to reduced pyridine nucleotides in mouse liver Advances in Experimental Medicine and Biology, 136 769-777
Data Type:	DNA damage in rat liver after i.p. exposure
HERO ID:	195226

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding of assessors was not reported.
	Metric 20:	Negative Control Response	High	× 1	1	The response of negative controls was appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake 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	Not Rated	NA	NA	Statistical analysis was not reported but may not be necessary for interpretation
	Metric 24:	Reporting of Data	Low	× 2	6	Results reported graphically without measure of variability
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.7	
Extracted			No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 103: **Animal toxicity evaluation results of Kitchin and Brown 1989 for acute hepatic DNA damage in rats**

Study Citation:	K. T. Kitchin, J. L. Brown (1989). Biochemical effects of three carcinogenic chlorinated methanes in rat liver Teratogenesis, Carcinogenesis, and Mutagenesis, 9(1,1), 61-69
Data Type:	Acute hepatic DNA damage in rats for CCl4
HERO ID:	195230

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride (CCl4).
	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	The test substance purity was not reported, but it was noted that it was "ACS grade".
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent solvent control groups were included (corn oil gavage).
	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 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	Preparation of the test substance was briefly reported. Storage of the test substance was not reported, but this is appropriate given the acute timeframe of the study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across 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	The exposure frequency and duration were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	The dose spacing was appropriate. The number of groups was somewhat lacking at 2. Rats were treated with 15, 70, 350, and 1050 mg/kg CCl4, but only the two highest doses were assessed for DNA damage. Furthermore, the two highest doses also showed increased ALT, an indicator of hepatotoxicity. This indicates that these doses caused cytotoxicity in addition to DNA damage, and that the findings of DNA damage at these doses are unreliable. Lower doses below the level that induces hepatotoxicity should have been assessed for DNA damage.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, age, and commercial source of the test animals were reported. Starting body weights of the test animals were not reported.

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Study Citation:	K. T. Kitchin, J. L. Brown (1989). Biochemical effects of three carcinogenic chlorinated methanes in rat liver Teratogenesis, Carcinogenesis, and Mutagenesis, 9(1,1), 61-69
Data Type:	Acute hepatic DNA damage in rats for CCl4
HERO ID:	195230

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported other than the number of rats per cage.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 8 for all groups).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It was not clear 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 outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative responses were observed in negative controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Starting body weight ranges were not included. Food and water consumption and respiratory rates were not reported, but this is appropriate given the study design.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No deaths or adverse health findings unrelated to the test compound were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by Bartlett's test for homogeneity of variance and Dunnett's multiple comparison test.
	Metric 24:	Reporting of Data	High	× 2	2	The data were reported adequately.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 104: **Animal toxicity evaluation results of Hachiya et al., 2000 study on mutagenicity in liver of MutaMouse**

Study Citation:	N. Hachiya, Y. Motohashi (2000). Examination of lacZ mutant induction in the liver and testis of Muta(TM)Mouse following injection of halogenated aliphatic hydrocarbons classified as human carcinogens Industrial Health, 38(2,2), 213-220
Data Type:	Mutagenicity in liver of MutaMouse
HERO ID:	202845

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	Low	× 1	3	Source of test substance was not reported.
	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	Medium	× 2	4	A negative control was used, however there are minor differences in treatment conditions.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control was not employed, however positive responses were elicited with other substances concurrently tested.
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was dissolved in olive oil however final concentration of prepared solution and storage conditions were not reported. Lack of information on storage unlikely to impact results since this was a single exposure study.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volumes and concentration of prepared test substance were not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses are provided clearly in Table 1 (700 or 1400 mg/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/duration (single exposure) were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Dose levels were not justified by authors, and results were judged to be negative; it is unclear if a higher dose would have elicited a positive response.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure (oral gavage) route was reported and appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal species, strain, sex, age, and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.
	Metric 15:	Number per Group	Low	× 1	3	2-3 animals/groups were tested. This number is lower than typically used for genotoxicity studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was cited elsewhere with virtually no additional details provided.

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Study Citation:	N. Hachiya, Y. Motohashi (2000). Examination of lacZ mutant induction in the liver and testis of Muta(TM)Mouse following injection of halogenated aliphatic hydrocarbons classified as human carcinogens Industrial Health, 38(2,2), 213-220
Data Type:	Mutagenicity in liver of MutaMouse
HERO ID:	202845

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment execution was cited elsewhere with no additional details provided.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	The number of phages scored was adequate but varied by group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable to this study.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was reported appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to treatment were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis by Fisher's exact test was reported; no additional details reported.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented in Table 1.
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 105: **Animal toxicity evaluation results of Sun et al., 2014 for a study on genomics/metabolomics outcomes**

Study Citation:	Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics Metabolomics, 10(6), 1293-1304
Data Type:	
HERO ID:	3487830

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	Commercial source was identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle (corn oil) controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not used for genomic/metabolomics alterations.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to each dose group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage were not described; however, omission of these details are unlikely to have a substantial impact on results (acute exposure).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Genomic/metabolic data provide mechanistic understanding for liver effects which occur after acute exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Adequate number of dose groups. Dose spacing justified by previous research.
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	FDA colony; species, strain and starting age reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	15/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mechanistic changes related to liver toxicity
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Summary data for metabolomics and genomics is provided for the high dose group only. Supplemental data tables are available for purchase.

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Study Citation:	Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics Metabolomics, 10(6), 1293-1304
Data Type:	
HERO ID:	3487830

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results.
	Metric 20:	Negative Control Response	High	× 1	1	Metabolomics changes were reported relative to control.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	Medium	× 2	4	Summary data is reported in the paper; supplemental data table are available for purchase.
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 106: **Animal toxicity evaluation results of Sasaki et al., 1998 study on DNA damage (comet assay) in multiple organs of mice exposed orally**

Study Citation:	Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y. Q. Su, N. Matsusaka, S. Tsuda (1998). Detection in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research, 419(1-3,1-3), 13-20
Data Type:	DNA damage (comet assay) in multiple organs of mice exposed orally
HERO ID:	5447470

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was Wako (Osaka, Japan).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Untreated mice were used as negative control instead of a gavage vehicle control. Authors note that their previous studies showed no difference in response between untreated and vehicle control groups. It is therefore, unlikely to have a substantial impact on results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not administered, however positive responses were elicited with other substances.
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance was prepared in olive oil, however final concentration of prepared substance was not reported, nor are the storage conditions.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volume is not reported. Furthermore, since concentration of prepared substance was not reported, the gavage volume cannot be inferred. Controls were not treated; however, the authors provided evidence that sham treatment would not alter the outcome
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (500, 1000, or 2000 mg/kg) without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency/ duration (single exposure) were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Maximum dose used was justified based on previous study (Sasaki et al., 1997).
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route (oral) was reported and appropriate; while not specified, it is inferred from the study that the method was gavage.
Domain 4: Test Organism						

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Study Citation:	Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y. Q. Su, N. Matsusaka, S. Tsuda (1998). Detection in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research, 419(1-3,1-3), 13-20
Data Type:	DNA damage (comet assay) in multiple organs of mice exposed orally
HERO ID:	5447470

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Male CD-1 mice, 7 wks old, were obtained from Charles River, Japan. Minor details (initial body weight and health status) are missing, but unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions (temperature, RH, light/dark cycle, and diet) were reported and adequate. Cage conditions were not reported but unlikely to substantially impact this short duration study
	Metric 15:	Number per Group	Medium	× 1	2	N=4; this number is slightly smaller than typical for this study type/outcome
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Assessment methodology is partially described and cited to other publications.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Outcome assessment execution is briefly reported. Controls were untreated; thus, the appropriate timing of outcome assessment is uncertain.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate (50 nuclei/organ/animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control data are presented in Table 1 and appear consistent across experiments.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake of study groups are not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	There were no deaths, morbidity, or clinical signs. No information on outcomes unrelated to exposure was reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods (1-way ANOVA and Dunnett test, $p<0.05$) are reported and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	Data for all groups, time points, and target organs are reported in Table 1 (mean±SEM).
Overall Quality Determination[‡]			Medium		1.8	
Extracted			Yes			

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Study Citation:	Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y. Q. Su, N. Matsusaka, S. Tsuda (1998). Detection in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research, 419(1-3,1-3), 13-20
Data Type:	DNA damage (comet assay) in multiple organs of mice exposed orally
HERO ID:	5447470

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 107: **In vitro evaluation results of Butterworth et al., 1989 for unscheduled DNA synthesis on primary hepatocytes**

Study Citation:	B. E. Butterworth, T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Jirtle, G. Michalopoulos, S. Strom (1989). Use of primary cultures of human hepatocytes in toxicology studies Cancer Research, 49(5,5), 1075-1084
Data Type:	UDS on primary hepatocytes - CCl4 and CHCl3
HERO ID:	6265

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were clearly identified as carbon tetrachloride and chloroform.
	Metric 2:	Test Substance Source	High	× 1	1	Test substances were purchased from Fischer.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported as ACS certified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included (media and DMSO controls).
	Metric 5:	Positive Controls	High	× 2	2	Although the study did not identify positive controls, many chemicals were included, several of which were strong genotoxic carcinogens. Two test substances (2-acetylaminofluorene and dimethylnitrosamine) are commonly used as positive control substances for a variety of genotoxicity assays and yielded positive responses in the present article.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were briefly described and cited elsewhere (Butterworth, et al., 1983, Butterworth, et al., 1987), but appeared ap-propriate for the outcome of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	It was reported that test substances were prepared "in DMSO or water" but it was not explicitly specified which test substances were dissolved in which. However, it was noted that the choice depended on solubility, so this is not considered to have substantially impacted results. Test substance storage was not reported, but the is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration are not reported; however, they are unlikely to have a substantial impact on results.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure doses were reported without ambiguity in Tables 2 and 3.

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Study Citation:	B. E. Butterworth, T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Jirtle, G. Michalopoulos, S. Strom (1989). Use of primary cultures of human hepatocytes in toxicology studies Cancer Research, 49(5,5), 1075-1084
Data Type:	UDS on primary hepatocytes - CCl4 and CHCl3
HERO ID:	6265

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration is reported to be between 18 to 24 hours. The exposure duration differed between different cultures of primary human hepatocytes isolated from different donors. This is not considered to have impacted results, as the donor samples were not directly compared to one another (i.e. statistics were conducted between treatment groups from a single donor, not comparing the responses of different donors to one another or pooling donor data).
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose spacing were appropriate. However, it was not clear that the doses selected were high enough to elicit UDS. When treatments elicited "pyknotic cells with missing cytoplasms and few grains", it was noted that this dose of test substance was considered "Toxic"; however, this was not noted for either CCl4 or CHCl3 in cells from any donor. Because negative results were obtained from all samples and it is not clear that the dose administered was high enough, this is considered to be unacceptable.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activators were not necessary since assays were performed in human and rat primary hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Details on primary human hepatocytes are given, including relevant donor information (age, sex, and reason for sample collection) and isolation and exposure methods. Details pertaining to primary rat hepatocytes are not provided; however, CCl4 and CHCl3 were not tested in rat hepatocytes.
	Metric 15:	Number per Group	Medium	× 1	2	Primary human hepatocytes were made from 4 different patients and assayed independently. The table legends denote footnotes indicating that 3 or 4 slides per dose level were utilized; however, the CCl4 and CHCl3 data do not have superscript letters indicating whether 3 or 4 slides were used per dose level. This is not considered to have substantially impacted results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate (150 cells per slide for Cases 12, 13, and 14; 100 cells per slide for Case 15). Although sampling was inconsistent between donors, this is not considered to have affected results, as the dose levels for each donor were sampled consistently, and the data from multiple donors were not directly compared or pooled.

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Study Citation:	B. E. Butterworth, T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Jirtle, G. Michalopoulos, S. Strom (1989). Use of primary cultures of human hepatocytes in toxicology studies Cancer Research, 49(5,5), 1075-1084
Data Type:	UDS on primary hepatocytes - CCl4 and CHCl3
HERO ID:	6265

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Automated measurements were made.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	The use of primary human cells introduces confounding variables (differences in age, sex and health of patients were noted). This, however, is unlikely to have a substantial impact on results, as these confounding variables were unavoidable for primary human samples and were controlled for appropriately; 1) there were several donors in the study, 2) details differentiating donor age/sex/health status and hepatocyte isolation were described, and 3) the data from multiple donors was not pooled or directly compared.
	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	Statistical methods were reported and appropriate.
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxic endpoints (description of pyknotic cells) were defined and appropriate.
	Metric 25:	Reporting of Data	Medium	× 2	4	The authors report that Table 3 contains typical results selected from several experiments. Data pertaining to CCL4 in rat hepatocytes is not included. It is unclear if the data were omitted or the assay was not run.
Overall Quality Determination‡			Unacceptable⋆⋆		1.3	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 108: **In vitro evaluation results of Garberg et al., 1988 for genotoxicity (alkaline elution) assay**

Study Citation:	Garberg, P; Akerblom, EL; Bolcsfoldi, G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution Mutation Research, 203(3), 155-176
Data Type:	Genotoxicity (Alkaline elution) assay
HERO ID:	7271

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance reported including manufacturer, but the batch/lot number not provided.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Although the authors did not report the purity of the chemical, it may be of a minor concern since the chemical is from a standard company.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Test authors report using a concurrent negative control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The authors do no report a concurrent positive control group. But it may not be a concern since they have used known genotoxic chemicals in this study.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	The QC part of this test criteria may not be applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses/concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups and concentration spacing were reported.
	Metric 13:	Metabolic Activation	High	× 1	1	Tests were done with and without metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The authors used a standard genotoxicity test model.
	Metric 15:	Number per Group	High	× 1	1	The authors reported the number of cells per group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent.
	Metric 18:	Sampling Adequacy	High	× 2	2	Adequate.

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Study Citation:	Garberg, P; Akerblom, EL; Bolcsfoldi, G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution Mutation Research, 203(3), 155-176
Data Type:	Genotoxicity (Alkaline elution) assay
HERO ID:	7271

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Low	× 1	3	The authors did not conduct statistical analysis.
	Metric 23: Data Interpretation	High	× 2	2	Data interpretation was consistent.
	Metric 24: Cytotoxicity Data	Unacceptable	× 1	4	Authors reported cytotoxicity data (cell viability).
	Metric 25: Reporting of Data	High	× 2	2	Data was reported for all doses.
Overall Quality Determination[‡]		Unacceptable[⋆⋆]		1.2	
Extracted		No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 109: **In vitro evaluation results of Sina 1983 for mutagenesis in rat hepatocyte assay**

Study Citation:	J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391
Data Type:	DNA damage (SSB) in rat hepatocytes for CCl4
HERO ID:	7323

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included. It was not specified whether the negative controls were treated with water, DMSO, or left untreated.
	Metric 5:	Positive Controls	High	× 2	2	Dimethylnitrosamine was utilized as a positive control in each assay. Positive results were obtained from positive control groups. This compound requires metabolic activation and was also utilized as a validation of hepatocyte metabolism.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well-described.
	Metric 7:	Standards for Tests	High	× 1	1	The QC criteria were adequate to demonstrate validity, acceptability, and reliability of this test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was reported. The storage of the test substance 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	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (3 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups and dose spacing was reported and appropriate.
	Metric 13:	Metabolic Activation	High	× 1	1	This assay did not include an exogenous metabolic activation step, as the cells used were primary rat hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity and origin of the test model were reported. No additional information was provided.
	Metric 15:	Number per Group	Low	× 1	3	The number of plates independently treated with CCl4 is not specified (although 2 replicates/plate was indicated). This may suggest the use of a single culture per concentration. .

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Study Citation:	J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391
Data Type:	DNA damage (SSB) in rat hepatocytes for CCl4
HERO ID:	7323

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	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 study replicate or group.
	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	High	× 1	1	Statistical analysis was not conducted. A three-fold increase in DNA single-strand breaks over negative controls was considered to be a positive result. Raw data are available for statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria (DNA single-strand breaks) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The cytotoxicity of all compounds tested was measured by either trypan blue dye exclusion or release of glutamate-oxaloacetate transaminase (GOT) from the cells. The methods were adequately described for each cytotoxicity assay, but it was unclear which assay was used for CCl4.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
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 110: **In vitro evaluation results of McCann et al., 1975 for Ames assay**

Study Citation:	J. McCann, E. Choi, E. Yamasaki, B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals Proceedings of the National Academy of Sciences, 72(12,12), 5135-5139
Data Type:	Ames assay
HERO ID:	8422

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The substance was identified by name as carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	Carbon tetrachloride was obtained from Mallinckrodt
	Metric 3:	Test Substance Purity	Low	× 1	3	Author stated that the purest grades available were used for all chemicals, but specific purity was not stated.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was either not included or not reported. Authors note the numbers of spontaneous revertant colonies (specific to each strain) that were subtracted from the numbers but these may be based on historical data.
	Metric 5:	Positive Controls	Not Rated	NA	NA	no positive control was used, but treatment-related positive responses were observed for other compounds
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods were cited to other publications and not well 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	Not Rated	NA	NA	Exposure methods cited to other publications with no details provided
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods cited to other publications with no details provided
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The highest test substance concentration tested was 10000 ug (10 mg) as reported in the table and footnotes
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Exposure methods cited to other publications with no details provided
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and concentration spacing were not reported, and dose-response was reportedly used to evaluate response.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Aroclor-induced rat liver S9 metabolic activation was used and is appropriate for the Ames assay, but the system was not described.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Test model reported along with limited descriptive information. S. typhimurium is standard model. Strains tested are reported inconsistently. Table indicates that two strains (TA100 and TA1535) were tested; however, footnotes report that nonmutagenic compounds were tested in at least 4 strains. Source of the test model was not reported.

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Study Citation: J. McCann, E. Choi, E. Yamasaki, B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals Proceedings of the National Academy of Sciences, 72(12,12), 5135-5139
Data Type: Ames assay
HERO ID: 8422

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Not Rated	NA	NA	Assay methods cited to another publication without additional details
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methods cited to another publication without additional details
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methods cited to another publication without additional details
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms or models 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	Low	× 1	3	Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Unacceptable	× 2	8	Evaluation criteria were not well described. In text, negative result is reported as <0.01 revertants/nmol, but in one table footnote, this is reported as the criterion for weakly mutagenic. Another footnote indicates that compounds were called non-mutagenic if there was no dose-response.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined and methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results. Footnotes indicate that compounds were tested "up to the maximum allowable concentration if the compound was inhibitory"; this may indicate testing to the limit of toxicity but it is not clear.
	Metric 25:	Reporting of Data	Low	× 2	6	Data reported as revertants per nmol or per plate. It is not clear which of 2 tested strains yielded the results.
Overall Quality Determination‡			Unacceptable⋆⋆		3.0	
Extracted			No			

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Study Citation:	J. McCann, E. Choi, E. Yamasaki, B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals Proceedings of the National Academy of Sciences, 72(12,12), 5135-5139
Data Type:	Ames assay
HERO ID:	8422

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 111: **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 CCl4					
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 carbon tetrachloride.
	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.
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; substantial toxicity was observed at the highest tested dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study design. The Saccharomyces cerevisiae cells used in the study contain cytochrome P-450, capable of converting chemicals to reactive products.
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						

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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 CCl4
HERO ID:	10054

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	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.
	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 112: **In vitro evaluation results of Uehleke et al., 1977 for mutagenicity assay in S. typhimurium**

Study Citation:	H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity Xenobiotica, 7(7,7), 393-400
Data Type:	mutagenicity assay in S. typhimurium
HERO ID:	10071

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as CCl4 and CHCl3.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported.
	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	Low	× 2	6	Study authors did report using a negative control (spontaneous mutation), but details regarding the negative control group were not reported
	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.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described, but appeared to be appropriate
	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	The test substance preparation was reported (dissolved in 5 ul of ethanol). The 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 the control and treated group
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in the results without ambiguity (8 mM). Doses for bacterial reverse mutation assays are usually reported in terms of ug/plate, but the volume of the incubation mixture (1.5 mL) for one plate is given, so ug/plate could be determined.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported and appropriate (60 minutes). Current standards indicate that 20 minutes is appropriate for the preincubation method, but this is not expected to have had a substantial impact on results.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Only one exposure concentration was tested. It was noted that the concentration used was determined in a preliminary experiment to not reduce survival of bacteria by more than 10%, but the range of doses found to produce cytotoxicity in this preliminary assay were not reported. Cytotoxicity was assessed concurrently with the present results, but results were not reported. It is not clear whether the dose utilized was high enough to detect a positive result.

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Study Citation: H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity Xenobiotica, 7(7,7), 393-400
Data Type: mutagenicity assay in S. typhimurium
HERO ID: 10071

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Low	× 1	3	Assays were conducted with metabolic activation described only as metabolically active rabbit liver microsomes (5 mg).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with limited descriptive information. The test model was routinely used for the outcome of interest. (S. typhimurium strains TA1535 and TA 1538). The source of the bacteria strains was not reported.
	Metric 15:	Number per Group	High	× 1	1	4 replicates per experimental condition were utilized.
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	Medium	× 1	2	Details on outcome assessment protocol execution were limited to determine if it was carried out consistently across the treated and control group.
	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	Statistics were not used to assess increased revertants/plate from the control. It was reported that the results was the mean of 4 different incubation mixtures, but the results for the different incubation mixtures was not reported. Standard deviations to the mean was not provided; however, the number of mutations was reported < 10. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable.
	Metric 23:	Data Interpretation	Unacceptable	× 2	8	Mutagenicity was expressed as cfu counted on the plates for his+ revertants per cfu of his- survivors; however, the evaluation criteria was not reported. The reporting of this ratio is inconsistent with current standards (absolute number of revertants per plate). Raw data is not reported to determine absolute number of revertants per plate. Therefore, this method of data interpretation and reporting is considered unacceptable.

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Study Citation:	H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity Xenobiotica, 7(7,7), 393-400
Data Type:	mutagenicity assay in S. typhimurium
HERO ID:	10071

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Survival rats of the test strains were determined, but results and methods of measurement were not reported. It was noted that the concentration used was determined in a preliminary experiment to not reduce survival of bacteria by more than 10%
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented for the control and the means of mutation frequency was reported (<10); the mutation frequencies for the 4 different incubation mixtures was not reported. Data for cytotoxicity was not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.1	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 113: **In vitro evaluation results of De Flora 1981 for mutagenicity assay in S. typhimurium**

Study Citation:	S. De Flora (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis, 2(4,4), 283-298					
Data Type:	mutagenicity assay in S. typhimurium					
HERO ID:	14322					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as carbon tetrachloride (CCl4) and formaldehyde (HCHO).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substances were reported (CCl4: Merck; HCHO: BDH). Lot/batch numbers were not reported, but these test substances are 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	Low	× 2	6	Study authors did report using a negative control (spontaneous mutations) for each strain, but it was unclear what the identity of the negative controls were (untreated or solvent).
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control was not specified but may not be required for this study.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described, but appeared to be appropriate. The methods section refer to original technical descriptions of the assays (Ames et al., 1975)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was inadequately reported ("dissolved and/or diluted either in twice distilled water or in [DMSO]"). This is not likely to have affected study results. 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 the control and treated group
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The upper test concentration limit was reported in the results with the limit dependent on Toxicity and based on preliminary data. However, the other doses tested for these compounds were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported; however, it was noted that the assay were performed according to the original technical description (Ames et al., 1975)
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Only one concentration (upper limit) was reported in the results. It was noted that solutions were diluted to a narrow range of concentrations below solubility or toxicity levels (based on preliminary data); only the upper limit was reported and the other tested concentrations were not reported. Therefore, the number of exposure groups and dose spacing were not reported.

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Study Citation: S. De Flora (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis, 2(4,4), 283-298
Data Type: mutagenicity assay in S. typhimurium
HERO ID: 14322

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (Aroclor-preteated rats liver S9 fractions). Some details regarding the metabolic activation system were not fully described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with limited descriptive information. The test model was routinely used for the outcome of interest. (S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100). The source of the bacteria strains was not reported.
	Metric 15:	Number per Group	High	× 1	1	It was noted that each concentration was tested in duplicate or triplicate.
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
	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	Statistics were not used to assess increased revertants/plate from the control. The number of net revertants at the top level of the dose-response curve and the mutagenic potency was calculated (revertants/nmol compound). 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	Evaluation criteria were reported; a key for the interpretation of the results is included in the paper.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Survival rats of the test strains were determined. Preliminary assays were conducted in or to determine toxicity to inform assay doses tested. The highest dose tested for CCl4 was based on its cytotoxicity level. Results for cytotoxicity were not reported.

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Study Citation:	S. De Flora (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis, 2(4,4), 283-298
Data Type:	mutagenicity assay in S. typhimurium
HERO ID:	14322

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Low	× 2	6	Data for the outcome was presented for the control (ranges of spontaneous mutations) and the result of the assays up to the highest dose tested were reported (with and without metabolic activation). Results for cytotoxicity and results for each tested dose group were not reported.
Overall Quality Determination‡		Unacceptable⋆⋆		2.0	
Extracted		No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 114: **In vitro evaluation results of De Flora et al., 1984 for bacterial reverse mutation (direct plate incorporation) S. typhimurium**

Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (direct plate incorporation) S. typhimurium
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as carbon tetrachloride and formaldehyde.
	Metric 2:	Test Substance Source	High	× 1	1	The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Ames reversion test: Study authors acknowledged using a concurrent negative control group, but it is unclear whether negative control was untreated or a vehicle solvent. Methods for this assay were partially cited to other publications that may contain this information. This is not expected to have substantially impacted results.
	Metric 5:	Positive Controls	High	× 2	2	Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-N-oxide, cyclophosphamide, and sodium azide) were included and yielded appropriate positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were briefly described; however, it was noted that the plate-incorporation test was conducted according to procedures described in Ames et al., 1975; the reported details suggest the assay was appropriate.
	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	The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, 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 the control and treated groups; applications were controlled for evaporation.

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (direct plate incorporation) S. typhimurium
HERO ID:	17980

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations tested were not reported; however, it is noted that various dilutions were performed by a geometric ratio of 2, starting with its solubility or toxicity limit. The highest dose tested can also be estimated based on the reported mutagenic potency value (potency (< 0.002 for CCl4 and < 0.08 for HCHO) was calculated by dividing the arbitrary value of 100 revertants by the nmoles corresponding to the maximum dose tested).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported; however, it was noted that the assay was performed according to the original technical description (Ames et al., 1975)
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of dose groups was not reported; however, it was noted that the compound was tested at various dilutions performed by a geometric ratio of 2 starting form its solubility or toxicity limit.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details, but not fully described; it was noted that the study was conducted according to Ames et al., 1975 where they describe the preparation of the S9 mix
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is routinely used for the outcome of interest (S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, TA97); sourced from Ames Lab (Department of Biochemistry, University of California, Berkely, CA).
	Metric 15:	Number per Group	Medium	× 1	2	It was noted that each concentration was tested in duplicate or triplicate.
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 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

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (direct plate incorporation) S. typhimurium
HERO ID:	17980

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Low	× 1	3	Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay. Mutagenic potency was calculated.
	Metric 23: Data Interpretation	High	× 2	2	Evaluation criteria were reported
	Metric 24: Cytotoxicity Data	Low	× 1	3	cytotoxicity data was not presented. It was noted that the doses tested were based on solubility or toxicity limit.
	Metric 25: Reporting of Data	Low	× 2	6	Data for exposure-related findings were presented qualitatively. Data for each study group was not reported. The result for the maximum dose tested; there were negative results for every strain tested at this dose. Data were not reported for controls.
Overall Quality Determination‡		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

Table 115: **In vitro evaluation results of De Flora et al., 1984 for bacterial reverse mutation (preincubation) in E. coli**

Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (preincubation) in E. coli
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Merck). The test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride. This is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The use of a solvent control was reported
	Metric 5:	Positive Controls	High	× 2	2	Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, and cyclophosphamide) were included and yielded appropriate positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were partially described; the reported details suggest the assay was appropriate.
	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	The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, 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 the control and treated groups; applications were controlled for evaporation.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Concentrations tested were not reported. Qualitative results were not associated with specific test concentrations.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose spacing was not reported

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (preincubation) in E. coli
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details not fully described; it was noted that the S9 mix was prepared according to Ames et al., 1975.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy).
	Metric 15:	Number per Group	High	× 1	1	It is noted that results were confirmed in at least 3 separate experiments.
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 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	Low	× 1	3	Statistical analysis was not conducted; independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	cytotoxicity was defined as survival; the assay evaluated and compared survival to a repair-deficient strain
	Metric 25:	Reporting of Data		× 2	NA	Data for exposure-related findings were presented qualitatively. Data for each study group was not reported. Qualitative results were not associated with specific test concentrations. Data were not reported for controls.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		1.8	
Extracted			No			

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Bacterial reverse mutation (preincubation) in E. coli
HERO ID:	17980

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 116: **In vitro evaluation results of De Flora et al., 1984 for DNA-repair test (liquid micromethod) in E. coli**

Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	DNA-repair test (liquid micromethod) in E. coli
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as carbon tetrachloride and formaldehyde.
	Metric 2:	Test Substance Source	High	× 1	1	The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors acknowledged that compounds were dissolved in water, DMSO, or ether, but did not specify that negative concurrent vehicle controls were utilized. Figure 1 depicts a plate map for each compound and is accompanied by test describing the mixture (media, bacteria, S9, test substance, etc.) that was added to each well of the plate. None of the wells in the plate map were a negative control. The responses were compared between repair-deficient bacteria and repair-proficient strains, so it is not clear that a concurrent negative vehicle control is required and this is considered acceptable. However, a concurrent vehicle control would help to ensure confidence in the reliability of results.
	Metric 5:	Positive Controls	Medium	× 2	4	Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-N-oxide, cyclophosphamide, and sodium azide) were included and appeared to yield positive results, although the criteria for a positive or negative result were not clearly defined.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were partially described with some omissions; however, it was noted that the procedure was similarly conducted as described by Kada et al., (1980) for the rec-assay; the reported details suggest the assay was appropriate. The study was designed to calibrate the method and support the use of this assay with the aid of other DNA-repair tests.

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Study Citation: S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type: DNA-repair test (liquid micromethod) in E. coli
HERO ID: 17980

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, 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 the control and treated groups; applications were controlled for evaporation.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Not all concentrations tested were reported; however, minimal inhibitory concentrations were reported in the results.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of dose groups was not reported; however, It was noted that the initial concentration of each compound was governed by its solubility or toxicity based on preliminary assays. The compounds were further diluted for a total of eight 2-fold dilutions.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details not fully described; it was noted that the S9 mix was prepared according to Ames et al., 1975.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy).
	Metric 15:	Number per Group	High	× 1	1	It was noted that each compound was assayed in at least 5 separate experiments to check reproducibility.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across 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						

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	DNA-repair test (liquid micromethod) in E. coli
HERO ID:	17980

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	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	Low	× 1	3	Statistical analysis was not conducted; independent statistical analysis is not possible.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were reported, but it was unclear what constituted a positive result.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	cytotoxicity data was not presented. It was noted that the doses tested were based on solubility or toxicity for bacteria inferred from preliminary assays
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented for each strain, but not for every dose tested. It is not clear if the values reported represent all replicate experiments. Data were not presented for controls.
Overall Quality Determination[‡]			~~Medium~~ ⟶ 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.

§ Evaluator's explanation for rating change: "There are a number of limitations to this study. All doses tested were not reported, statistical analysis was not conducted, and the reporting of results was limited."

Table 117: **In vitro evaluation results of De Flora et al., 1984 for spot test in E. coli**

Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Spot test in E. coli
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as carbon tetrachloride and formaldehyde.
	Metric 2:	Test Substance Source	High	× 1	1	The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Testing of a negative untreated or solvent control for the spot test was not reported. The responses were compared between repair-deficient bacteria and repair-proficient strains, so it is not clear that a concurrent negative vehicle control is required and this is considered acceptable. However, a concurrent vehicle control would help to ensure confidence in the reliability of results.
	Metric 5:	Positive Controls	High	× 2	2	Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, and sodium azide) were included and yielded appropriate positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were partially described; the reported details suggest the assay was appropriate.
	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	The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, 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 the control and treated groups; applications were controlled for evaporation.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Concentrations tested were not reported. Qualitative results were not associated with specific test concentrations.

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Spot test in E. coli
HERO ID:	17980

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose spacing was not reported
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The spot assay was conducted only in the absence of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy).
	Metric 15:	Number per Group	Unacceptable	× 1	4	The assay was repeated only if no inhibition was detected.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across 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	Low	× 1	3	Statistical analysis was not conducted; independent statistical analysis is not possible. However, statistical analysis is not necessarily required for this assay.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	cytotoxicity endpoints were not defined and it cannot be determined if cytotoxicity was accounted for in the interpretation of the study results.
	Metric 25:	Reporting of Data	Unacceptable	× 2	8	Data for exposure-related findings were presented qualitatively. Data for each study group was not reported. Qualitative results were not associated with specific test concentrations. Data were not presented for controls.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.1	

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Study Citation:	S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198
Data Type:	Spot test in E. coli
HERO ID:	17980

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

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 118: **In vitro evaluation results of Zeiger et al., 1988 for Salmonella mutagenicity assay**

Study Citation:	E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans (1988). Salmonella mutagenicity tests: IV: Results from the testing of 300 chemicals Environmental and Molecular Mutagenesis, 11(Suppl 12,Suppl 12), 1-158
Data Type:	Salmonella mutagenicity assay for CCl4
HERO ID:	24516

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified as carbon tetrachloride. A CASRN was also provided (56-23-5).
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was identified as a manufacturer (J.T. Baker Chemical). 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	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were concurrently run.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were run concurrently and elicited a positive response (at least a two-fold increase in revertants).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were partially described partially cited to other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in DMSO. Storage was not reported, but was not expected to have an impact on the assay.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across the study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in Tables 58.1 and 58.2.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate (20 minutes followed by 2 days incubation).
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure groups and dose spacing was justified. Half-log dose intervals up to toxic dose (initially determined) were used in this study.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Study authors reported exposures were conducted in the presence of metabolic activation and the type and source and concentration in final culture were described. However, preparation was cited to another publication.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Cell lines were obtained from Dr. Bruce Ames (University of California, Berkeley). These strains are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each dose was tested in triplicate.
Domain 5: Outcome Assessment						

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Study Citation:	E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans (1988). Salmonella mutagenicity tests: IV: Results from the testing of 300 chemicals Environmental and Molecular Mutagenesis, 11(Suppl 12,Suppl 12), 1-158
Data Type:	Salmonella mutagenicity assay for CCl4
HERO ID:	24516

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for this study.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcomes were assessed by automated measurements. It is noted that the study indicated that chemicals were coded (laboratories were not aware of the identity of the chemical being tested).
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 and procedures.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data are presented as means ±SEM (statistical analysis not required by study type).
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	An initial toxicity assay was performed to determine the dose range for the mutagenicity assay. Toxicity was defined as concentrations that decreased the number of his+colonies, caused a clearing of the density of the background lawn, or both. The method was cited to another publication.
	Metric 25:	Reporting of Data	High	× 2	2	Data were shown 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 119: **In vitro evaluation results of Simmon et al., 1977 for bacterial reverse mutation**

Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258					
Data Type:	Bacterial reverse mutation for CCl4					
HERO ID:	29451					
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 (carbon tetrachloride).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported incompletely (reported as a commercial supplier). Since the test substance is not expected to vary in composition and the test substance was obtained from a commercial supplier, the omitted details are unlikely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity and grade of test substance were not reported ("reagents of the highest available purity"). It was indicated that purity was not determined for most chemicals. This is not expected to have impacted results, as the test substance was obtained from a commercial supplier.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A concurrent negative control (solvent control) was used, but the control response was not described.
	Metric 5:	Positive Controls	Low	× 2	6	A concurrent positive control was used (unnamed), but the control response was not described.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited in another publication (Ames et al., 1975), but appeared to be appropriate for the assays in desiccators (bacteria) and in suspension (yeast); some details (e.g., cell density for the bacterial assay) were reported incompletely. Special test conditions were used to account for the volatility of the test substance.
	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 test substance preparation was reported (e.g., test chemical added to glass petri plate for bacterial assay); lack of storage conditions are not likely to substantially impact the study results given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported or inferred from the text with few study details (or cited to Ames et al., 1975). Exposures were reportedly for "7 to 10 hours" (unclear if time varied among concentrations or different chemicals tested); however, these differences were not expected to substantially affect the study results.

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Study Citation: V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258
Data Type: Bacterial reverse mutation for CCl4
HERO ID: 29451

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The amounts of test substance used were not reported. It was stated that a "wide range of doses was tested up to 5 mg/plate or a dose which gave a toxic response, whichever was lower."
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Exposure duration was reported (7-10 hours for the bacterial assay, 4 hours for the yeast assay). The duration of these assays was presumed appropriate for the study type (given that positive responses were observed for the test chemical or other chemicals used in the study). It is possible that the exposure duration for the bacterial assay (7 to 10 hours) varied across dose levels. It is also possible that the variation in exposure duration was across test substances instead, so this metric is still considered acceptable.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported. The study states (for all chemicals) that a wide range of doses were tested up to a given concentration or until a toxic concentration was achieved (whichever was lower).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system (e.g., rat or mice liver cells cited to Ames et al., 1975) was re-ported in the study; however, some details were not described. These omissions are unlikely to have a substantial impact on the results.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. Reporting limitations are unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates per study group were not reported (though procedures were reportedly consistent with Ames et al., 1975). Because there are no error bars in any graphs, it is considered likely that only one plate per dose was included in the study design, which is lacking.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	It is not clear that the exposure duration (7-10 hr) and post-exposure incubation time ("approximately" 40 hrs) were equal for all doses of a test substance.
	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	This metric is not applicable to this study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258
Data Type:	Bacterial reverse mutation for CCl4
HERO ID:	29451

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) identified 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	Statistical analysis was presumably not conducted, and insufficient information was provided for independent statistical analysis (data not reported quantitatively or graphically for CCl4 due to negative response, as determined by study authors). However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported and the omissions are likely to have a substantial impact on interpretation of the results.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were evaluated, but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data		× 2	NA	Data presentation was inadequate (no data was shown).
Overall Quality Determination‡			Unacceptable⋆⋆		2.6	
Extracted			No			

⋆⋆ Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 120: **In vitro evaluation results of Brams et al., 1987 for Ames and SOS Chromotest**

Study Citation:	A. Brams, J. P. Buchet, M. C. Crutzen-Fayt, C. De Meester, R. Lauwerys, A. Leonard (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure) Toxicology Letters, 38(1-2,1-2), 123-133
Data Type:	Ames and SOS Chromotest for CCl4
HERO ID:	51352

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 carbon tetrachloride (CCl4).
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was purchased from a manufacturer (Merck). A lot number was also provided.
	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 negative (vehicle-only) controls.
	Metric 5:	Positive Controls	High	× 2	2	The study reported using reference mutagens for the Ames assay (2-NF and NaN3 as direct mutagens and 2-AA as an indirect mutagen). For the SOS chromotest. 4-nitroquinoline oxide was used as a direct mutagen.
	Metric 6:	Assay Procedures	Medium	× 1	2	For the Ames assay, most of the methods/procedures are described, but another publication is also cited (Maron and Ames 1983). For the SOS chromotest, methods were partially described and also cited to the procedure recommended by the manufacturer of the kit (Orgenics).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicated that only freshly prepared solutions were tested. The lack of information on storage is not likely to impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be administered consistently across study groups (both assays).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A range of doses was provided. For the Ames assay, 3 concentrations from 100 to 1000 ug/plate were tested; for the SOS chromotest, 7 dilutions from 15.4 ng/mL to 1.54 mg/mL were tested. At least two concentrations were reported without ambiguity for each assay.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study types (Ames test 48 hours; SOS chromotest 2 hours).

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Study Citation: A. Brams, J. P. Buchet, M. C. Crutzen-Fayt, C. De Meester, R. Lauwerys, A. Leonard (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure) Toxicology Letters, 38(1-2,1-2), 123-133
Data Type: Ames and SOS Chromotest for CCl4
HERO ID: 51352

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	For the Ames assay, concentrations of 100 to 1000 ug/plate were tested (3 concentrations); for the SOS chromotest, 15.4 ng/mL to 1.54 mg/mL were tested (at least 7 dilutions). The study indicated that doses for the Ames test were chosen based on previously published data.
	Metric 13:	Metabolic Activation	Medium	× 1	2	For the Ames assay, the method of preparing metabolic activator was mainly cited to another publication (Maron and Ames, 1983). For the SOS chromotest, the metabolic activator was included in kit. For the Ames assay, it was indicated that the protein content of S9 was checked, and the sterility of the S9 mix was controlled in each assay.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Salmonella typhimurium strains TA 97, TA 98, and TA 100 were obtained from Prof. Ames (it was indicated that strains were checked weekly for their genotypes). Bacteria for the SOS chromotest were supplied in the kit. These test systems are appropriate for these outcomes of interest.
	Metric 15:	Number per Group	Medium	× 1	2	The study indicated that the Ames test was performed in duplicate with 3 plates/concentration. The number per group was not clearly specified for the SOS chromotest (but the assay was performed according to manufacturer's instructions).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was reported for each assay and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments were consistently applied across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for this study.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	Confounding variables in the test design were not reported. The study indicated that great care was taken to ensure the consistency of the bacterial strains used in the Ames assay. The same lot of the test substance was used across all study groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Confounding variable in outcomes unrelated to exposure were not reported. For the Ames assay, the study indicated that plates were carefully examined to detect contamination; based on control cultures, the viable counts in the bacterial cultures were consistent.
Domain 7: Data Presentation and Analysis						

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Study Citation:	A. Brams, J. P. Buchet, M. C. Crutzen-Fayt, C. De Meester, R. Lauwerys, A. Leonard (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure) Toxicology Letters, 38(1-2,1-2), 123-133
Data Type:	Ames and SOS Chromotest for CCl4
HERO ID:	51352

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	There is no indication that statistical analyses were performed. The study indicates that for the Ames assay, data were evaluated as the mean +/- SD of 3 plates (not shown). For the SOS chromotest, quantitative measurements were obtained using a photometer.
	Metric 23:	Data Interpretation	Medium	× 2	4	For the Ames assay, the evaluation criteria were specified (at least a 2-fold increase in revertants in one strain, a dose-response, and a reproducible effect), For the SOS chromotest, the evaluation criteria are not clearly specified (based on quantitative photometer measurements and based on manufacturer's recommendations).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	For the Ames study, it was indicated that the background lawn of the plates was examined to detect toxic effects; the study results indicate that viable counts in the bacterial cultures were very similar (no additional information provided). For the SOS chromotest, it was indicated that a viability control was performed at each dilution of the test substance; no toxicity was reported for CCl4.
	Metric 25:	Reporting of Data	Medium	× 2	4	Negative data are reported qualitatively. No quantitative data for CCl4 are provided in the study report.
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 121: **In vitro evaluation results of Nakamura et al., 1987 for DNA repair**

Study Citation:	S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type:	DNA repair for CCl4
HERO ID:	51515

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 carbon tetrachloride.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Katayama chemical). Although a batch/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/grade of the test substance was not reported. However, it was indicated that chemicals were of the highest quality commercially available.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were reported; however, it is not clear if they were run concurrently with test substance (e.g., DMSO was one of the 151 chemicals tested in the assay).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive responses were observed for several of the 151 chemicals tested in this study (demonstrating that the test is capable of detecting a positive response) it is unclear if these were run concurrently with test substance. It is noted the list of chemicals tested included test substances used as positive controls in the Ames assay.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were briefly described and partially cited to another publication (Oda et al., 1985).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Based on the text and the properties of the test substance, it can be inferred that the test substance was prepared in DMSO (but this was not explicitly stated). Storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Since responses were negative for all doses tested, only the highest dose was reported (5300 ug/mL).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (2 hours) and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and/or spacing was not reported. Only the highest tested dose was reported (no rationale provided).

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Study Citation: S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type: DNA repair for CCl4
HERO ID: 51515

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	Method of preparing liver S9 fraction are only partially reported (i.e., prepared from rats pretreated with phenobarbital and 5,6-benzoflavone).
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The cell line was developed in house; limited details were provided. The system was described as novel (not yet routinely used to assess this outcome).
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates per group was not indicated.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methods for outcome assessment were largely cited to another publication (Miller, 1972).
	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 metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Binding was not necessary for this study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variable were reported in test design/procedure.
	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	Not Rated	NA	NA	Data are presented qualitatively (i.e., reported as negative). Statistical analyses do not appear to have been performed (despite the use of the term 'significant' in the results section), but are not required by study type (fold changes can be used to evaluate the response).
	Metric 23:	Data Interpretation	High	× 2	2	It was indicated that a 2-fold increase in beta-galactosidase activity above background levels was considered a positive effect. The study authors further classified chemicals used in the study as potent inducers (6-fold changes), intermediate inducers (3-fold changes), or weak inducers (2-fold changes).
	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	Only one data point is reported (highest tested concentration); however, since the results were negative, this is unlikely to have a substantial impact on results.

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Study Citation:	S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type:	DNA repair for CCl4
HERO ID:	51515

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Overall Quality Determination[‡]		Unacceptable[⋆⋆]		1.9	
Extracted		No			

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 122: **In vitro evaluation results of Direnzo et al., 1982 for DNA adducts**

Study Citation:	A. B. Direnzo, A. J. Gandolfi, I. G. Sipes (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA Toxicology Letters, 11(AMST,AMST), 243-252
Data Type:	DNA adducts for CCl4
HERO ID:	75145

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 [radiolabeled] carbon tetrachloride.
	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 radiochemical purity of the test substance (>99%) was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	This metric is not required by study type, as the measurement of radiolabeled test compound is the outcome. However, heat-denatured microsomes were used as incubation blanks in this study.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	High	× 1	1	The methods and assay procedures were sufficiently described. It is noted that another publication was cited to the methods section as well (Sipes and Gandolfi 1980).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (mixed with unlabeled test substance and diluted in ethanol). 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.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (60 min) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	A single concentration was tested, but was appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The activation system used (hepatic microsomal protein obtained form phenobarbital-treated rats) was appropriate; details of isolation/preparation were cited to other publications.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model, calf thymus DNA, was appropriate for the outcome of interest. The source of calf thymus DNA was reported (a manufacturer).

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Study Citation:	A. B. Direnzo, A. J. Gandolfi, I. G. Sipes (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA Toxicology Letters, 11(AMST,AMST), 243-252
Data Type:	DNA adducts for CCl4
HERO ID:	75145

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The experiment with microsomal bioactivation was repeated six times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate/sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently.
	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.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design/procedures were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	The study indicated that protein contamination could influence results. The authors took steps to specifically remove protein contamination from the isolated DNA.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The mean and standard deviation (and including n, the number of experiments performed) were reported and were appropriate for the study type.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not explicitly stated (other than detection of radiolabeled test substance bound to DNA), however, the study reported test substances that covalently bound to DNA at levels exceeding 0.3 nmol/mg DNA.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design, as no cells were utilized.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data for the radiolabeled CCl4 group were reported adequately. Data for binding to untreated DNA was not shown.
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 123: **In vitro evaluation results of Perocco and Prodi 1981 for scheduled and unscheduled DNA synthesis**

Study Citation:	P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218					
Data Type:	Scheduled and unscheduled DNA synthesis for CCl4					
HERO ID:	75278					
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 carbon tetrachloride.
	Metric 2:	Test Substance Source	Medium	× 1	2	The donor source and commercial source of the various test substances tested in this study were identified, but it was unclear which test substance originated from which source.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of the test substance was identified to be between 97-99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included (DMSO).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance was reported. The storage of the test substance 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 final concentrations of the test substance used in the experiments was reported without ambiguity (in uL/mL).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (4 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported (3 treatment groups plus control). Results for two of the three treatment groups were obtained from a representative toxicity experiment; subsequent experiments used a single dose. The concentrations selected in the representative assay were not useful for evaluating a dose-response. The study indicates that the test substance induced toxicity at tested concentrations.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Rat liver phenobarbital-induced S9 mix was utilized. More detailed methods regarding metabolic activation were cited to other references.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, no further information regarding gender, age, or other important demographics were included.

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Study Citation: P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218
Data Type: Scheduled and unscheduled DNA synthesis for CCl4
HERO ID: 75278

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	It was reported that six replicates were used per experimental condition.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment 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.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, it is unclear whether the 6 replicates for each experimental condition originated from 6 individual donors. It is also unclear whether different experimental conditions were tested on the same set of lymphocytes (e.g. Dose 1 tested on lymphocytes originated from donors A, B, and C; Dose 2 tested on lymphocytes originating from donors D, E, and F; etc).
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistical analysis was not conducted and raw data were not provided, preventing an independent statistical analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not explicitly specified.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Scheduled DNA synthesis (SDS) was used as a measure of toxicity. Methods used to determine SDS were reported; however, cytotoxicity endpoints were not well-defined (i.e., the response that constituted a toxic effect).
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported by exposure group; however, data for experiments conducted with and without activation were not reported separately.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		1.8	
Extracted			No			

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Study Citation:	P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218
Data Type:	Scheduled and unscheduled DNA synthesis for CCl4
HERO ID:	75278

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 124: **In vitro evaluation results of Loveday et al., 1990 for chromosome aberrations and sister chromatid exchanges**

Study Citation:	K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303
Data Type:	SCEs and CAs for CCL4
HERO ID:	106324

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 carbon tetrachloride. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial supplier 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 (>99%) was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Both media and solvent controls were used with each assay.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls both with activation (cyclophosphamide) and without activation (mitomycin C) were used. A low- and high-dose positive control was used for the SCE assay.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described for SCE and CA experiments.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the 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 and preparation of stock solutions), but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information regarding exposure administration was reported and it appears that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Some of the doses/concentrations (including the highest test dose eliciting a negative response) were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for each assay was reported; however, the time of exposure varied based on activation status, assay, and cell cycle delay.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose selections were described in detail.The highest dose in the SCE assay was based on solubility or toxicity (or 5 mg/mL if there were no issues). Doses the CA assay were based on cell cycle delay and toxicity noted in SCE assay. The study indicates that, for the SCE assay, dilutions were made to achieve 10 test concentrations in a half-log series covering a range of five logs;and at least 5 concentrations were spaced using two merged half-log scales for the CA assay.

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Study Citation:	K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303
Data Type:	SCEs and CAs for CCL4
HERO ID:	106324

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	The study authors reported exposures were conducted in the presence of metabolic activation and the type and source rat liver microsomal fraction) and volume in final culture were reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (CHO cells) was described in detail and were considered appropriate for the endpoints assessed. These cells are routinely used in assays of this type.
	Metric 15:	Number per Group	Medium	× 1	2	The number of replicates was not clearly indicated. It was noted that tests were repeated to confirm positive results if there was one or more elevated point, or if toxicity was too great.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	For each assay, the outcome assessment methodology was described in detail and appropriate for the outcome 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 data in the appendix indicate that 1000+ chromosomes per concentration were evaluated for SCEs; 200 cells were evaluated per concentration for CAs (fewer than recommended by study type).
	Metric 19:	Blinding of Assessors	High	× 1	1	The samples were reported to be coded and assessed by a single person.
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	High	× 1	1	It was indicated that cells were found to be free of mycoplasma for all experiments.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical analyses were described and appropriate for data described. Data analysis was partially cited to other publications.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive/negative result were described. For SCEs, a trend test and a 20% increase over solvent was designated as positive. For CAs, a positive response was defined as p (adjusted) < 0.05 based on analyses of increases in CAs over solvent controls.

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Study Citation:	K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303
Data Type:	SCEs and CAs for CCL4
HERO ID:	106324

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 24: Cytotoxicity Data	Medium	× 1	2	The study indicated that toxicity was determined as the percent of confluence of the cell monolayer in treated flasks in comparison with control flasks. Although measurements of toxicity were not fully reported, the summary table indicates doses above which toxicity was observed (when applicable; i.e., at 1490 ug/mL in the SCE assay for CCl4 without activation). The study indicates that toxicity was taken into account for these assays (highest dose evaluated was the one that allowed sufficient cells for analysis).
	Metric 25: Reporting of Data	Medium	× 2	4	Data are shown for some (but not all) dose groups (based on the number of dose groups that were reportedly used in each assay).
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 125: **In vitro evaluation results of Schiestl et al., 1989 for intrachromosomal recombination**

Study Citation:	R. H. Schiestl, R. D. Gietz, R. D. Mehta, P. J. Hastings (1989). Carcinogens induce intrachromosomal recombination in yeast Carcinogenesis, 10(8,8), 1445-1455
Data Type:	Intrachromosomal recombination for CCl4
HERO ID:	188190

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test material was clearly identified as carbon tetrachloride.
	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	Low	× 1	3	Test substance purity/grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study specifically indicated that solvent controls were used, and that this group was treated the same way as the other concentrations. For a few chemicals used in the study (other than CCl4), the solvent is specified as acetone or DMSO; it is inferred that water was the solvent for other chemicals (including CCl4).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. However, several known mutagens (including radiation) and substances that elicited positive results in carcinogenicity and/or Ames assays were tested and gave positive results. It was not explicit that these conditions were intended to serve as positive controls for this assay.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods/procedures were described in detail. The volume of the test concentrations added to incubation tubes was not stated.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Use of a standard is not required for this test method.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	It could be inferred that the test substance was dissolved in solvent. It was indicated that tubes were sealed. However, the stability of the test substance in that solvent (presumably water) was not demonstrated. Storage was not reported (but is not likely 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 were reported without ambiguity in Table II.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (17 hours) and appeared to be appropriate for the outcome of interest.

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Study Citation: R. H. Schiestl, R. D. Gietz, R. D. Mehta, P. J. Hastings (1989). Carcinogens induce intrachromosomal recombination in yeast Carcinogenesis, 10(8,8), 1445-1455
Data Type: Intrachromosomal recombination for CCl4
HERO ID: 188190

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (4 plus controls). A rationale for dose selection was not provided. Spacing may not have been appropriate based on toxicity and the absence of a clear dose-response.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type (all experiments were conducted in the absence of S9).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (Saccharomyces cerevisiae diploid strain RS112) used was described (with respect to genotypic features). The methods used to construct this strain in the lab were reported (not from a commercial source).
	Metric 15:	Number per Group	High	× 1	1	Experiments were performed in triplicate with two plates per test concentration.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment was adequately described. However, it is not clear that the assessment was sensitive to the outcome of interest because increased intrachromosomal recombination was seen only in the presence of substantial toxicity (89% to 99%).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The study indicated that data derived from less than 5 colonies were not included. Based on data presented in Table II, the colony yield (HIS+) ranged from 232 to 8 across the range of doses tested.
	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 variables in test design or procedures 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	Not Rated	NA	NA	Statistical analysis was not performed (and not required); data were evaluated as fold-increase over controls.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive response were specified. A minimum increase of 2-fold over the spontaneous frequency in a dose-dependent manner was regarded as evidence of inducibility.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cell viability was included; the authors did not discuss the potential impact of cytotoxicity on the observed results. Other than cell survival (as a percent), the authors did not define the cytotoxicity endpoint.

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Study Citation:	R. H. Schiestl, R. D. Gietz, R. D. Mehta, P. J. Hastings (1989). Carcinogens induce intrachromosomal recombination in yeast Carcinogenesis, 10(8,8), 1445-1455
Data Type:	Intrachromosomal recombination for CCl4
HERO ID:	188190

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 25: Reporting of Data	Medium	× 2	4	Data were reported by exposure group (without a measure of variance). The study indicated that the same qualitative results were obtained in other strains.
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 126: **In vitro evaluation results of Beddowes et al., 2003 for DNA SSB (Comet assay), M1dG and 8-oxodG adducts**

Study Citation:	E. J. Beddowes, S. P. Fau, J. K. Chipman (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress Toxicology, 187(2-3,2-3), 101-115					
Data Type:	DNA SSB (comet assay), M1dG and 8-oxodG adducts,					
HERO ID:	194414					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The substance was identified by chemical name, no CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	A commercial source (Sigma-Aldrich) for the test substance 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	An untreated negative control was included. CCL4 was administered as a vapor so untreated control is appropriate
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls were used; however positive responses were observed
	Metric 6:	Assay Procedures	High	× 1	1	Adequate assay procedures were described and appropriate for the outcomes of interest.
	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	Medium	× 1	2	Test substance was applied using gas-tight syringes and cells culture in sealed flasks to minimize volatilization. Test substance storage was not reported but unlikely to affect this short term study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	The study reports that the method of administration was intended to allow the test substance to be absorbed as a vapor in a uniform manner. Volumes applied across groups were not reported. Measures were consistently taken to minimize evaporation prior to administration.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Initial concentrations (0.25, 1, and 4 mM) were estimated using partition coefficients calculated for hepatocytes using the headspace-vial equilibrium technique.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (2hrs) was clearly reported and was appropriate for the outcomes of interest
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of nonzero exposure groups (3) was appropriate and adequate to evaluate a dose-response. Cytotoxicity was statistically significant at the highest dose (92% of control).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The study used primary hepatocytes, metabolic activation was not required.
Domain 4: Test Model						

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Study Citation:	E. J. Beddowes, S. P. Fau, J. K. Chipman (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress Toxicology, 187(2-3,2-3), 101-115
Data Type:	DNA SSB (comet assay), M1dG and 8-oxodG adducts,
HERO ID:	194414

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (primary hepatocytes from female Wistar rats) was adequately described including cell isolation procedures and tests for cell viability . The model was appropriate for the outcomes of interest; authors noted that female rats are sensitive to CCL4 carcinogenicity. The number of rats used was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The study indicates five separate experiments (comet assay), threeseparate experiments (8-oxodeoxyguanosine measurements), and five separate experiments (M1dG concentrations) were used. It is unclear whether "separate experiments" indicates replicates from the same pool of isolated hepatocytes, or if this represents true biological replicates (separate populations of cells from different animals).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were reported and sensitive to the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent assessment across groups was inferred from the descriptions
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number of cells/exposure were reported, but the study does not indicate the number of cells evaluated used for each endpoint. 100 comets randomly assessed per slide for comet assay.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No blinding was reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study identified cytotoxicity as a potential confounder for measurements of DNA SSB, however this was not indicated as a major concern for CCL4. The study identified apoptosis as a potential confounder, but indicated that the Comet assay can effectively distinguish between DNA SSB and DNA fragmentation from apoptosis.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No disproportionate outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Details of statistical analysis were not provided, however, the statistical methods used (ANOVA, student T-test) were reported in figure legends and were appropriate. Means and SEM can be derived from the (graphical) data provided.
	Metric 23:	Data Interpretation	High	× 2	2	Scoring/evaluation for most endpoints was based on obtaining statistical significance. Category assignment for the degree of DNA damage was done according to a prior publication.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The method(s) for determining cytotoxicity (LDH release and MTT) were reported and appropriate.

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Study Citation:	E. J. Beddowes, S. P. Fau, J. K. Chipman (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress Toxicology, 187(2-3,2-3), 101-115
Data Type:	DNA SSB (comet assay), M1dG and 8-oxodG adducts,
HERO ID:	194414

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 25: Reporting of Data	High	× 2	2	Data were adequately reported for all outcomes and exposures.
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 127: **In vitro evaluation results of Selden et al., 1994 unscheduled DNA synthesis assay in rat hepatocytes**

Study Citation:	J. R. Selden, F. Dolbeare, J. E. Miller, J. H. Clair, K. Mcgettigan, J. A. Dijohn, G. A. Dysart, J. G. Deluca (1994). Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes Mutation Research, 315(2,2), 147-167
Data Type:	UDS assay in rat hepatocytes
HERO ID:	194433

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified as carbon tetrachloride, CASRN provided
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Aldrich) is provided
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity is not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study included a solvent (DMSO) negative control.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were reported (MMS and 2-AAF)
	Metric 6:	Assay Procedures	High	× 1	1	UDS was evaluated using two methods (flow cytometric and autoradiographic). Assay procedures for each method were adequately 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	Unacceptable	× 1	4	Preparation and storage were not described, except that CCL4 was dissolved in DMSO. Cells were cultured and treated in petri dishes, and the study did not describe any steps taken to reduce/prevent volatilization from the dishes.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Details of application (e.g., volumes added) were not provided. The study did not report any measures taken to inhibit/prevent volatilization from petri dishes.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A concentration range (1-10ˆ-2mM) was reported, but individual test concentrations and the exact number of test groups is not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported to be 18-20hrs.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exact number of exposure groups was not specified, but the text indicates that typical doses varied by half-log increments and the dose range was given. The maximum concentration, selected based on range-finding studies, was cytotoxic, exceeded solubility limits, or exceeded 10 mM.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not necessary because the study was performed in primary rat hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (primary hepatocytes from male Crl:CD(SD)BR rats aged 6-9 wks) was appropriate for the outcomes assessed.

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Study Citation:	J. R. Selden, F. Dolbeare, J. E. Miller, J. H. Clair, K. Mcgettigan, J. A. Dijohn, G. A. Dysart, J. G. Deluca (1994). Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes Mutation Research, 315(2,2), 147-167
Data Type:	UDS assay in rat hepatocytes
HERO ID:	194433

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Single assays were done on CCL4. For FCM, the assay was reportedly run in triplicate. For ARG assays yielding negative results, the highest nontoxic dose was analyzed to confirm.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The purpose of the study was to evaluate and validate a novel DNA repair assay (FCM) by comparing it to a conventional autoradiography assay. The methodologies were adequately described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	It is unclear if there were any differences in the timing of outcome assessment across study groups because exposure duration was reported as a range. No other potential differences in outcome assessment were identified.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	For the standard ARG study, 30 cells/slide were counted from 2-3 slides per dose. This may be lower than the recommend 50 cells/culture using six cultures, unless otherwise justified. For FCM, 1,000 cells/replicate were collected which may be lower than the typical 2,000 cells/replicate for other flow cytometry applications, however the lower sample sizes are not expected to have a great impact on study results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not applicable to the flow cytometric analysis. Cells in the ARG assay were counted using an automated colony counter and therefore blinding is not necessary.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	Initial conditions were reported and consistent across groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	A number of confounding variables related to the methods were recognized and discussed specific to flow cytometry (autofluorescence, low yields, high debris), and to the ARG method (cell-to-cell variability within a culture and culture-to culture variability within a dose. These were considered in statistical analysis and in the interpretation of the data.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis was used
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were adequately reported.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The study tested cytotoxicity, which was adequately defined (< 90% viability of concurrent control).
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were presented for lowest effective response (ARG) and maximal and lowest effective responses (FCM).
Overall Quality Determination‡			Unacceptable⋆⋆		1.4	

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Study Citation:	J. R. Selden, F. Dolbeare, J. E. Miller, J. H. Clair, K. Mcgettigan, J. A. Dijohn, G. A. Dysart, J. G. Deluca (1994). Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes Mutation Research, 315(2,2), 147-167
Data Type:	UDS assay in rat hepatocytes
HERO ID:	194433

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

[⋆⋆] Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 128: **In vitro evaluation results of Simmon and Tardiff 1978 for Ames assay**

Study Citation:	V. F. Simmon, R. G. Tardiff (1978). The mutagenic activity of halogenated compounds found in chlorinated drinking water 2 417-431					
Data Type:	Ames Assay					
HERO ID:	194442					
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 carbon tetrachloride
	Metric 2:	Test Substance Source	Medium	× 1	2	The reagents were reported to be purchased from commercial suppliers, but the specific sources were not provided
	Metric 3:	Test Substance Purity	Medium	× 1	2	Reagents were reported to be of the "highest available purity" however specific purities were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative solvent (DMSO) controls were included in each experiment
	Metric 5:	Positive Controls	Low	× 2	6	Positive controls were reported to be known mutagens that either do or do not require metabolic activity, but the specific substances used were not provided and results were not reported.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were cited to another reference but some details were partially described. The assay procedure generally follows usual practices.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Information on preparation was not complete, as it is not clear whether CCL4 was tested as a liquid or vapor. There was no information on storage; however the study duration was short. The study indicated measures were taken to account for test substance volatility (studies performed in sealed desiccators)
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details were not reported (e.g., amount of test substance, whether administered as vapor or liquid).
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Exposure concentrations were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Unacceptable	× 2	8	Exposure duration was not reported clearly.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups was not reported
	Metric 13:	Metabolic Activation	Low	× 1	3	S9 metabolic activation was reported with no descriptive details and the species source was not provided
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is routinely used for the outcome of interest. The source of the test model was reported (Gift from B. Ames)

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Study Citation: V. F. Simmon, R. G. Tardiff (1978). The mutagenic activity of halogenated compounds found in chlorinated drinking water 2 417-431
Data Type: Ames Assay
HERO ID: 194442

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	The number of test strains (2) is less than current standards however, the experiment was repeated at least once and the number of replicates (2/strain) per experiment was acceptable.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was not reported, but may have been described in the cited publication.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methodology was not reported, but may have been described in the cited publication.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study design
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study design
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Numbers of cells in initial inoculates were not reported and could significantly impact the results
	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	Statistical analysis was not reported; however statistical analysis is not always necessary for Ames assay interpretation.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and evaluation criteria were not reported but may have been provided in the cited publication
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it does not appear that cytotoxicity was considered in the study interpretation.
	Metric 25:	Reporting of Data	Unacceptable	× 2	8	Data presentation for CCL4 was inadequate. The only information provided was a statement indicating that CCL4 was not mutagenic
Overall Quality Determination‡			Unacceptable**		3.1	
Extracted			No			

** Consistent with the *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one 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 129: **In vitro evaluation results of Sivikova et al., 2001 for CA assay in ovine lymphocytes**

Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	CA assay in ovine lymphocytes
HERO ID:	194444

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as carbon tetrachloride, no CASRN reported.
	Metric 2:	Test Substance Source	High	× 1	1	A commercial source (Microchem, Bratislava, Slovak Republic) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99.8%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study included a solvent (DMSO) control
	Metric 5:	Positive Controls	High	× 2	2	Concurrent and appropriate positive control (ethylmethanesulphonate) was used
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided that are presumed to provide more methodological 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	Low	× 1	3	Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could effect the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (48 hrs) was reported. It is presumed that this duration is acceptable for this test model system.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index).

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Study Citation: K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type: CA assay in ovine lymphocytes
HERO ID: 194444

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Low	× 1	3	Metabolic activation was not included for this endpoint and no positive responses were reported. Metabolic activation was included in other assays reported in the same study. In is unclear why metabolic activation was not included for CAs.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old)
	Metric 15:	Number per Group	High	× 1	1	Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Low	× 2	6	Text indicates that 100 well-spread metaphases were analyzed for CAs; however, Table 1 reports that 200 metaphases were determined per concentration. Guidance recommends 300 per concentration to conclude that a test chemical is clearly negative.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Use of coded slides or blinded evaluations were not specified.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of cells 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	High	× 1	1	Statistical analysis (<U+03C7>2-test) was appropriate for the outcome of interest and adequately described. Data were reported as Means with SD
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Mitotic index was evaluated and reported for each exposure concentration. High concentration was selected to achieve at least 50% reduction in mitotic index. For CCL4, high concentration MI was 1.35 compared with 2.3 in controls.
	Metric 25:	Reporting of Data	Medium	× 2	4	Results were reported for all experiments and groups as mean and SD percent breaks. The number of metaphases evaluated is reported inconsistently in the study.

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Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	CA assay in ovine lymphocytes
HERO ID:	194444

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Overall Quality Determination[‡]		~~High~~ $\longrightarrow$ Medium[§]		~~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.

§ Evaluator's explanation for rating change: "CCL4 was not tested for CA in presence of metabolic activation. Tests for SCE and MN in the same study were performed with and without metabolic activation; for these endpoints, metabolic activation was not required to achieve positive results in this system. It is not clear why S9 was not included in the test for CA. Results without activation can be considered valid under the conditions of the study, but may not yield a complete picture of the potential for CAs."

Table 130: **In vitro evaluation results of Sivikova et al., 2001 for MN assay in ovine lymphocytes**

Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142					
Data Type:	MN assay in ovine lymphocytes					
HERO ID:	194444					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as carbon tetrachloride, no CASRN reported.
	Metric 2:	Test Substance Source	High	× 1	1	A commercial source (Microchem, Bratislava, Slovak Republic) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99.8%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study included a solvent (DMSO) control
	Metric 5:	Positive Controls	High	× 2	2	Concurrent and appropriate positive controls (mitomycin C without S9 and cyclophosphamide with S9) were used in each experiment
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided and presumed to provide more methodological 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	Low	× 1	3	Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could affect the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure durations (48hrs without and 2hrs with metabolic activation) were reported. It is presumed that these durations are acceptable for this test model system.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index).

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Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	MN assay in ovine lymphocytes
HERO ID:	194444

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	A commonly used metabolic activation system ((Aroclor 1254 induced male rat S9) was used. Details on the source, method of preparation, and volume in culture were provided.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old)
	Metric 15:	Number per Group	High	× 1	1	Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	× 2	4	Text reports that MN were evaluated in a total of 1000 binucleated cells per concentration but table 2 reports that 2000 binucleate cells per concentration were determined. Guidance recommends 2000.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Use of coded slides or blinded evaluations was not specified but is recommended for this endpoint.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of cells 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	High	× 1	1	Statistical analysis (<U+03C7>2-test) was appropriate for the outcome of interest and adequately described. Data were reported as Means with SD
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not concurrently evaluated for this assay, however cell survival was evaluated with other assays using the same cell source and test concentrations.
	Metric 25:	Reporting of Data	High	× 2	2	Results were reported for all experiments and groups.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	MN assay in ovine lymphocytes
HERO ID:	194444

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 131: **In vitro evaluation results of Sivikova et al., 2001 for SCE assay in ovine lymphocytes**

Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142					
Data Type:	SCE assay in ovine lymphocytes					
HERO ID:	194444					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as carbon tetrachloride, no CASRN reported.
	Metric 2:	Test Substance Source	High	× 1	1	A commercial source Microchem, Bratislava, Slovak Republic) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99.8%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study included a solvent (DMSO) control
	Metric 5:	Positive Controls	High	× 2	2	Concurrent and appropriate positive controls (mitomycin C without S9 and cyclophosphamide with S9) were used in each experiment
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided and presumed to provide more methodological 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	Low	× 1	3	Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could effect the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure durations (48 hrs without and 2 hrs with metabolic activation) were reported. It is presumed that these durations are acceptable for this test model system.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index).

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Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	SCE assay in ovine lymphocytes
HERO ID:	194444

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	High	× 1	1	A commonly used metabolic activation system (Aroclor 1254 induced male rat S9) was used. Details on the source, method of preparation, and volume in culture were provided.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old)
	Metric 15:	Number per Group	High	× 1	1	Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was well-reported and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	× 2	2	The total number of metaphases evaluated for SCE (50) is adequate
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Use of coded slides or blinded evaluations were not specified.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of cells 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	High	× 1	1	Statistical analysis (ANOVA) was appropriate for the outcome of interest and adequately described. Data were reported as means with SD
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was concurrently evaluated as proliferation index
	Metric 25:	Reporting of Data	High	× 2	2	Results were reported for all experiments and groups.
Overall Quality Determination[‡]			High		1.4	
Extracted			Yes			

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Study Citation:	K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
Data Type:	SCE assay in ovine lymphocytes
HERO ID:	194444

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 132: **In vitro evaluation results of Tafazoli et al., 1988 for MN assay**

Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	MN assay
HERO ID:	194476

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified as Carbon tetrachloride and CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source was reported
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent media-alone controls were used (no solvents were used during test preparation)
	Metric 5:	Positive Controls	Low	× 2	6	Appropriate positive controls for conditions with and without metabolic activation were used. The data indicates that one replicate of the positive control in the presence of metabolic activation was toxic and the outcomes for this sample could not be evaluated (a separate positive control - same substance, same concentration, different donor - was able to be sampled fully - 2000 cells). The study did not discuss the reasoning for the observed cell death.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were clearly described and appropriate for the outcome of interest.
	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	Low	× 1	3	Information on test substance preparation was adequately described. Methods were employed (use of sealed bottles) to prevent evaporation during the process. The duration of the test substance preparation however was lengthy (48hours, shaking at 37 degrees), and the rationale for this and the potential impact on stability was not discussed. There is further uncertainty about the stability of the test substance due to lack of DMSO as a solvent.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Consistent application methods are inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Initial Test substance concentrations (4 per donor) were reported without ambiguity. Analytical concentrations measured after the media preparation procedure were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest (3hrs with activation, 48hrs without)
	Metric 12:	Exposure Route and Method	High	× 1	1	A wide range of concentrations were tested up to a cytotoxic dose and were appropriate for the outcome of interest.

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Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	MN assay
HERO ID:	194476

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Low	× 1	3	The study included conditions of metabolic activation (s9), however, the source and method of preparation of the S9 mix were not provided.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (primary human lymphocytes) was appropriate. Descriptive information on the source and method of isolation was provided.
	Metric 15:	Number per Group	High	× 1	1	Two replicates (cultures at each concentration from each donor) were utilized. This is adequate according to current standards and guidelines for in vitro MN assays.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across exposure groups
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was generally adequate for the outcome of interest (2000+ cells per treatment group, except in the case of toxicity).
	Metric 19:	Blinding of Assessors	High	× 1	1	The study reports coded slides were used.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	It was unclear why the samples from two individual donors were treated with different doses. However, the individual donors were analyzed independently, so this is not considered to have substantially impacted results.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables on outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were clearly stated and appropriate for the outcome of interest.
	Metric 23:	Data Interpretation	High	× 2	2	Circumstances yielding a positive result were described. Positive results were based on reaching statistical significance.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The relative division index was considered a concurrent measure of cytotoxicity
	Metric 25:	Reporting of Data	High	× 2	2	Data was adequately presented across all groups
Overall Quality Determination[‡]			High		1.3	
Extracted			Yes			

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Study Citation:	M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential Mutagenesis, 13(2,2), 115-126
Data Type:	MN assay
HERO ID:	194476

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor.

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

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

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

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

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

7 Developmental and Reproductive

Table 133: **Animal toxicity evaluation results of Schwetz et al., 1974 for a study on inhalation developmental toxicity study in rats**

Study Citation:	Schwetz, BA; Leong, BKJ; Gehring, PJ (1974). Embryo- and fetotoxcitiy of inhaled carbon tetrachloride 1,1-dichloroethane and methyl ethyl ketone in rats 28(1,1), 452-464
Data Type:	Inhalation developmental toxicity study in rats
HERO ID:	3675473

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reagent grade CCl4
	Metric 2:	Test Substance Source	High	× 1	1	Source and lot number were reported. Lot No. 9256, Burdick & Jackson Lab, Inc., Muskegon, Michigan
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%; listed an unknown component of 0.1%; determined by gas-liquid chromatography
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Control animals for each experiment exposed concurrently to filtered room air
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not rated/applicable for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study 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 method and equipment used to generate the test substance as a vapor was reported and appropriate; storage conditions were not reported
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	nominal concentrations were reported and vapor concentration was measured analytically in the chamber and reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	7 hr/day GD 6-15
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two vapor concentrations tested; one concentration in an initial experiment and the other in a second experiment. Each experiment had its own control group. It is not clear if these experiments were conducted concurrently.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Adult, Sprague Dawley female rats; starting body weight was reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were reported, but conditions were not specific for temperature, humidity, and light cycle.

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Study Citation:	Schwetz, BA; Leong, BKJ; Gehring, PJ (1974). Embryo- and fetotoxcitiy of inhaled carbon tetrachloride 1,1-dichloroethane and methyl ethyl ketone in rats 28(1,1), 452-464
Data Type:	Inhalation developmental toxicity study in rats
HERO ID:	3675473

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type
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	The outcome assessment methodology addressed or reported the intended outcomes of interest
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling for the outcomes of interest were adequate; developmental endpoints were evaluated for litters.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable; initial pathology review; no other subjective outcomes were assessed
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group was adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study reported minor differences among the study groups with respect to food consumption of dams; however, there was no effect on the conception rate or number of implantations or size of litters.
	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
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were not specified in the methodology section of the paper, but are statistical tests used were specified and clear in the results tables.
	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		1.3	
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.

8 Mechanistic

Table 134: **Animal toxicity evaluation results of Yasuda et al., 2000 study on DNA fragmentation (TUNEL assay)**

Study Citation:	M. Yasuda, T. Okabe, J. Itoh, S. Takekoshi, H. Hasegawa, H. Nagata, R. Y. Osamura,Watanabe K (2000). Differentiation of necrotic cell death with or without lysosomal activation: application of acute liver injury models induced by carbon tetrachloride (CCL4) and dimethylnitrosamine (DMN) Journal of Histochemistry and Cytochemistry, 48(10,10), 1331-1339
Data Type:	DNA fragmentation (TUNEL assay) for CCl4
HERO ID:	194648

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 formula.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Controls were not given corn oil vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of concern.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in corn oil was described; storage was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Information was presented for dose calculation (% in solution, mL/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single gavage dose was adequate for the outcome.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	A single dose was adequate for the outcome. Dose justification was not reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Intraperitoneal injection is an appropriate route, but not environmentally relevant.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, age and starting body weight was provided. A commercial source was reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group was fewer than typically used in studies of this type (2-3/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment method reported and was senditive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assess consistently.

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Study Citation:	M. Yasuda, T. Okabe, J. Itoh, S. Takekoshi, H. Hasegawa, H. Nagata, R. Y. Osamura,Watanabe K (2000). Differentiation of necrotic cell death with or without lysosomal activation: application of acute liver injury models induced by carbon tetrachloride (CCL4) and dimethylnitrosamine (DMN) Journal of Histochemistry and Cytochemistry, 48(10,10), 1331-1339
Data Type:	DNA fragmentation (TUNEL assay) for CCl4
HERO ID:	194648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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.
	Metric 20:	Negative Control Response	High	× 1	1	Controls responded appropriately (no positive TUNEL staining).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake were not reported for each group.
	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	Not Rated	NA	NA	Figures presented histology and microscopy sections. Data were not quantitaive.
	Metric 24:	Reporting of Data	High	× 2	2	Figures presented both confocal laser scanning microscopy (CLSM) and electron microscopy results.
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.