United States
Environmental Protection Agency

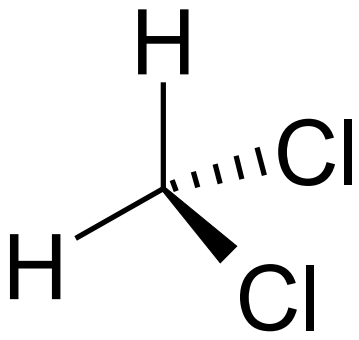
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Methylene Chloride

Systematic Review Supplemental File:

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

CASRN: 75-09-2



June 2020

Table Listing

Acute (<24 hr) and Short-term (1-30 days)

Other

Subchronic (30-90 days)

Chronic (>90 days)

Genetic toxicity studies

Developmental and **Reproductive**

1 Acute (< 24 hrs) and Short-term (1-30 days)

Table 1: **Animal toxicity evaluation results of Aranyi et al 1986 for a 3-hour and 5-day inhalation immunotoxicity study on hematological and immune outcomes**

Study Citation:	Aranyi, C; O'Shea, WJ; Graham, JA; Miller, FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses Fundamental and Applied Toxicology, 6(4,4), 713-720					
Data Type:	3-hour and 5-day inhalation immunotoxicity study					
HERO ID:	61922					
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 SMILES
	Metric 2:	Test Substance Source	Medium	× 1	2	Commercial source was identified (B&J laboratories; omitted details include the batch/lot number.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance purity was not reported, but not expected to be of concern
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	filtered air; a control group was used, but lacks some details that are unlikely to have a substantial impact on results.
	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	Low	× 1	3	The preparation of the test substances for the inhalation chamber was generally described for all substances, but not specific for this test substance. There was no information on the storage of the test substance.
	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	reported target and actual test concentration s
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	exposure frequency and duration of exposure were identified; a single 3-hour exposure or 3 hours/day for a 5-day exposure is not standard for this study type.

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Study Citation:	Aranyi, C; O'Shea, WJ; Graham, JA; Miller, FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses Fundamental and Applied Toxicology, 6(4,4), 713-720
Data Type:	3-hour and 5-day inhalation immunotoxicity study
HERO ID:	61922

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only 1 dose tested. The number of exposure concentrations and dose spacing was justified by study authors; "when significant effects were found in single exposures at the TLV level or above exposure, the concentration was reduced stepwise until a no-measurable-effect level was reached for a single exposure; this dose was then used for the 5-day exposure
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance; a dynamic whole-body chamber was used for vapors
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	4-5 wk old Female CD1 mice
	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	I7 to 24 mice per treatment
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	incomplete reporting of minor details of the outcome assessment protocol, but unlikely to have a substantial impact on results; few specific details on how the ratio of viable bacterial counts to the radioactive counts and the determination of bactericidal activity were conducted.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	The study did not report whether assessors were blinded to treatment group, 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	Low	× 2	6	Initial body weight and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	

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Study Citation:	Aranyi, C; O'Shea, WJ; Graham, JA; Miller, FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses Fundamental and Applied Toxicology, 6(4,4), 713-720
Data Type:	3-hour and 5-day inhalation immunotoxicity study
HERO ID:	61922

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 2: **Animal toxicity evaluation results of Dow 1988 for an acute inhalation study on neurological/behavior, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	Dow Chemical Company (1988). Initial submission: Evaluation of the acute neuropharmacologic effects of dichloromethane in rats (final report) with attachments and cover letter dated 050792					
Data Type:	Acute inhalation					
HERO ID:	4214025					
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 and lot number were provided. Analytical verification of the test substance was performed by infrared spectroscopy.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported (99.97% as reported by source, and 99.94%, as determined by gas chromatography).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using an appropriate concurrent negative control group (exposed to filtered air) for some of the tests (e.g., Probe 3); however, other tests did not have a true negative control group (e.g., were pre-exposed to DCM for 3 days [conditioning phase] and were then exposed to filtered air on 4th day).
	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	Medium	× 1	2	Preparation methods of the test substance were reported and were suitable for the test substance. Storage methods were not reported but this is not considered to have a substantial impact on the results for this acute study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The study authors reported adequate details of exposure administration and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Nominal and target chamber concentrations were reported with mean and standard deviations. The analytical method used to measure chamber concentrations (IR spectrometry) was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and were appropriate for this study type (i.e., acute toxicity).

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Study Citation: Dow Chemical Company (1988). Initial submission: Evaluation of the acute neuropharmacologic effects of dichloromethane in rats (final report) with attachments and cover letter dated 050792
Data Type: Acute inhalation
HERO ID: 4214025

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	There were minor limitations regarding the concentration spacing. Only one concentration was tested in each of the probe studies (e.g., 2000 ppm or 4000 ppm) and in each study effects were observed on neurological measures.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance. Whole-body chamber exposures were used, rather than nose- or head-only exposures, but this appears to be acceptable for DCM, which was exposed as a vapor and not expected to condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Some test animal characteristics (source, species, strain, body weight, and sex) were reported; however, age and health status prior to testing was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions (target conditions for temperature, humidity, light-dark cycle) were reported and were adequate and the same for the control and exposed animal groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group was low in some tests (e.g., with 4000 ppm, there were only two animals/group), but some tests used 8 animals/group. Overall the number ranged from 2-8 animals.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was reported, but some details of the methodology were unclear due to incomplete reporting. (e.g., COHb measurement).
	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 in all study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control responses were reported for the outcomes of interest and were adequate.
Domain 6: Confounding / Variable Control						

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Study Citation:	Dow Chemical Company (1988). Initial submission: Evaluation of the acute neuropharmacologic effects of dichloromethane in rats (final report) with attachments and cover letter dated 050792
Data Type:	Acute inhalation
HERO ID:	4214025

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported; however, DCM is a potential respiratory irritant but respiratory rate measurement was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure and data on attrition and/or health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and were appropriate for the datasets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for evaluated outcomes by exposure group. Individual data values were provided in appendices.
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 3: **Animal toxicity evaluation results of Moser et al 1995 for a 1 to 14-day oral neurotoxicity study on neurological/behavior, mortality, and body weight outcomes**

Study Citation:	Moser, VC; Cheek, BM; Macphail, RC (1995). A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity Journal of Toxicology and Environmental Health, Part A: Current Issues, 45(2), 173-210
Data Type:	1 to 14-d oral neurotoxicity
HERO ID:	76020

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified as analytical grade dichloromethane
	Metric 2:	Test Substance Source	Medium	× 1	2	Aldrich Chemical Co; batch no. not reported
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle controls (corn oil)
	Metric 5:	Positive Controls	Medium	× 1	2	In some neurobehavioral testing positive controls are needed/suggested. This study did not include a positive control; however, results from 10 different compounds were reported, with at least one compound showing positive effects in each neurofunctional domain tested. THis suggests validity of the test.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Assigned to test groups using random stratification tables based on body weights (nonrandom component).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	DCM was mixed with corn oil for gavage; storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent across groups; 10 ml/kg dose volume
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose selection based on acute LD50 values. Acute (1 d): 0, 3, 10, 30, or 56% of LD50 (0, 101, 337, 1012, 1889 mg/kg) Subacute (14 d):0, 1, 3, 10 or 30% of LD50 (0, 34, 101, 337, 1012 mg/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	1 or 14 d
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	4 exposures plus control
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Adult female F344 rats

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Study Citation:	Moser, VC; Cheek, BM; Macphail, RC (1995). A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity Journal of Toxicology and Environmental Health, Part A: Current Issues, 45(2), 173-210
Data Type:	1 to 14-d oral neurotoxicity
HERO ID:	76020

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Consistent between groups. Adequate reporting of conditions.
	Metric 15:	Number per Group	Medium	× 1	2	8/group. Numbers are acceptable but given variability in neurobehavioral endpoints, more animals/group would be ideal.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Neurological: FOB and motor activity at several time-points; baseline established prior to exposure Mortality, BW Note: Systemic effects (organ weight, serum chemistry, urinalysis, histopathology) were evaluated in these rats; however, results of systemic analysis reported in separate study (Berman et al. 1995)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were assessed for relevant outcomes
	Metric 19:	Blinding of Assessors	High	× 1	1	All testing was performed blind.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported; baseline values similar between groups
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Baseline FOB and motor testing was reported and results were comparable between groups. Decreased BW of unknown magnitude was reported in the two highest dose groups (steady weight loss).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	dose-by-time interaction ANOVA
	Metric 24:	Reporting of Data	Medium	× 2	4	Mortality reported in text. Most neurobehavioral findings with significant effects were reported graphically; remaining were reported qualitatively. Body weight loss reported qualitatively.
Overall Quality Determination[‡]			High		1.3	
Extracted			Yes			

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Study Citation:	Moser, VC; Cheek, BM; Macphail, RC (1995). A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity Journal of Toxicology and Environmental Health, Part A: Current Issues, 45(2), 173-210
Data Type:	1 to 14-d oral neurotoxicity
HERO ID:	76020

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 4: **Animal toxicity evaluation results of Warbrick et al 2003 for 28-day inhalation immunotoxicity study**

Study Citation:	Warbrick, E.V., Kilgour, J.D., Dearman, R.J., Kimber, I., Dugard, P.H. (2003). Inhalation exposure to methylene chloride does not induce systemic immunotoxicity in rats Journal of Toxicology and Environmental Health, Part A: Current Issues, 66(13,13), 1207-1219
Data Type:	28-day inhalation immunotoxicity study
HERO ID:	732101

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified, but not characterized further
	Metric 2:	Test Substance Source	High	× 1	1	Source was identified: Merck Ltd. (Poole, Dorset, UK)
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	air alone; Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Positive Controls	High	× 1	1	cyclophosphamide; chemical is recommended by the U.S. EPA as a positive control for immunotoxicity studies in which the integrity of antibody production is examined
	Metric 6:	Randomized Allocation	High	× 1	1	Rats were randomized into groups according to body weight
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	test substance preparation was reported, but storage conditions were not; deficiencies in reporting not likely to have a substantial effect on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Exposure was therefore maintained within ± 6.7% of the target of 5000ppm; GC was used to measure chamber test substance and vehicle concentration; overall achieved mean concentration for the study was 5187 + - 347 ppm
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 hours/day, 5 days/week for 28 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Only one dose tested, but justified the decision to use a single high dose as a screening study because there have been no indications of immunotoxic effects in a number of animal 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
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Young adult (8–12wk old, 154–177 g) male and female Sprague-Dawley (SD) rats

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Study Citation:	Warbrick, E.V., Kilgour, J.D., Dearman, R.J., Kimber, I., Dugard, P.H. (2003). Inhalation exposure to methylene chloride does not induce systemic immunotoxicity in rats Journal of Toxicology and Environmental Health, Part A: Current Issues, 66(13,13), 1207-1219
Data Type:	28-day inhalation immunotoxicity study
HERO ID:	732101

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported
	Metric 15:	Number per Group	Medium	× 1	2	8/sex
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	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	outcomes of interest were not subjective measurements
	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	Low	× 2	6	The respiratory rate was no measured for the inhalation exposure. Methylene chloride is expected to be a respiratory irritant.
	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	High	× 1	1	Statistical methods were clearly described and appropriate for the dataset
	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 presentation of the results and statistics
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 5: **Animal toxicity evaluation results of General et al 1976 for a 14-day oral study in rats on mortality, nutrition and nutrition and metabolic/adult exposure body weight, neurological/behavior, gastrointestinal, and respiratory outcomes**

Study Citation:	General Electric Company (1976). Dichloromethane fourteen day range finding study in rats					
Data Type:	14-day oral - rat					
HERO ID:	4213647					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (CASRN and name provided).
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was reported (p. 5), but the chemical description, including source, may not be totally accurate according to p. 5, so there are some uncertainties about the source.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group (received the vehicle via gavage).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not indicated by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how the animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance preparation and storage conditions were not sufficiently reported and this may have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency and duration were reported; although administration was only 14 days in this repeated-dose study, the study was designed to be a range-finding study for a longer-duration exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and dose spacing were considered adequate to address the purpose of the study; however, the selection of dose levels was not justified by the study authors (e.g., basis for selection was not stated).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method (oral gavage) were reported and were suited to the test substance.
Domain 4: Test Organism						

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Study Citation: General Electric Company (1976). Dichloromethane fourteen day range finding study in rats
Data Type: 14-day oral - rat
HERO ID: 4213647

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, sex, and starting body weight were reported; however, the source, health status, and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions (e.g., temperature, humidity, light-dark cycle) were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals per study group (5/sex/group) was lower than the typical number used in studies of the same or similar type (i.e., repeated-dose studies).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	This repeated-dose study only evaluated mortality, general behavior, appearance, body weight, food consumption, and gross pathology, with no additional evaluation of endpoints typically evaluated in studies of similar type (e.g., histopathology); however, it was designed to be a range-finding study.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There is insufficient information to evaluate whether outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups regarding confounding variables.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were not reported and insufficient data were reported to allow independent analysis (e.g., necropsy results appear to be incompletely reported).

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Study Citation:	General Electric Company (1976). Dichloromethane fourteen day range finding study in rats
Data Type:	14-day oral - rat
HERO ID:	4213647

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group (e.g., gross necropsy), but results were described in the text and data were only reported for some outcomes.
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 6: **Animal toxicity evaluation results of General et al 1976 for a 14-day oral study in dogs on mortality, nutrition and nutrition and metabolic/adult exposure body weight, neurological/behavior, gastrointestinal, and respiratory outcomes**

Study Citation:	General Electric Company (1976). Dichloromethane fourteen day range finding study in dogs					
Data Type:	14-day oral - dog					
HERO ID:	4213648					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (CASRN and name).
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was reported (p. 5), but the chemical description, including source, may not be totally accurate according to p. 5, so there are some uncertainties about the source.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported (e.g., whether also dosed with vehicle) and the lack of details is likely to have a substantial impact on results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not indicated by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how the animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance preparation and storage conditions were not sufficiently reported and this may have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency and duration were reported; although administration was only 14 days in this repeated-dose study, the study was designed to be a range-finding study for a longer-duration exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and dose spacing were considered adequate to address the purpose of the study; however, the selection of dose levels was not justified by the study authors (e.g., basis for selection was not stated).

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Study Citation: General Electric Company (1976). Dichloromethane fourteen day range finding study in dogs
Data Type: 14-day oral - dog
HERO ID: 4213648

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method (oral gavage) were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, sex, and starting body weight were reported; however, the source, health status, and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions (e.g., temperature, humidity, light-dark cycle) were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred.
	Metric 15:	Number per Group	Low	× 1	3	The number of animals per study group was insufficient to characterize toxicological effects (1 animal/sex/group). Therefore, results can only be used as support to other studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	This repeated-dose study only evaluated mortality, general behavior, appearance, body weight, food consumption, and gross pathology, with no additional evaluation of endpoints typically evaluated in studies of similar type (e.g., histopathology); however, it was designed to be a range-finding study.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There is insufficient information to evaluate whether outcomes were assessed consistently across study groups. (e.g., no information on whether evaluations were conducted at same time of day or on the same day of week).
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups regarding confounding variables.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	The number of animals per group was not conducive to statistical analysis.

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Study Citation:	General Electric Company (1976). Dichloromethane fourteen day range finding study in dogs
Data Type:	14-day oral - dog
HERO ID:	4213648

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex.
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~2.0~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

§ Evaluator's explanation for rating change: "The study was downgraded to low (from medium) because the number of dogs evaluated per dose for each outcome is too limited to provide confidence in evaluating dose-response results. However, the results of this range-finding study can be consulted, as needed, when considering the body of animal toxicity results."

Table 7: **Animal toxicity evaluation results of Shell Oil 1986 for a 10-day inhalation study in rat and mice on hepatic and respiratory outcomes**

Study Citation:	Shell Oil Company (1986). Ten day inhalation toxicity study to investigate the effects on rat and mouse liver and lung with methylene chloride
Data Type:	10-day inhalation, rat mice, liver and lungs
HERO ID:	4213825

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source was reported and measurement of concentration levels were conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	The grade and purity were provided and such that any effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required for this study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	The Latin square method was used for animal allocation (re: obtaining similar body weights/group).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The methods and equipment used were described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The analytical method used to measure test atmospheres was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The duration and frequency were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The concentrations were based on results from lifetime inhalation studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	The inhalation chamber was appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, source, age, and initial body weight were reported. the health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported and the same for the groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcomes of interest.

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Study Citation:	Shell Oil Company (1986). Ten day inhalation toxicity study to investigate the effects on rat and mouse liver and lung with methylene chloride
Data Type:	10-day inhalation, rat mice, liver and lungs
HERO ID:	4213825

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to the outcomes in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control responses were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	DCM is a respiratory irritant and respiratory rate was not measured.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No differences were reported or inferred but health outcomes not discussed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Student's t-test was used for some data, but histopathological and electron microscopic findings were not analyzed.. Data were available to conduct an independent analysis.
	Metric 24:	Reporting of Data	Medium	× 2	4	Quantal and continuous data were reported for the outcomes of interest. Severity incidences were reported for some endpoints.
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

2 Other

Table 8: **In vitro evaluation of Schenk et al 2018 for skin permeability**

Study Citation:	L. Schenk, M. Rauma, M. N. Fransson, G. Johanson (2018). Percutaneous absorption of thirty-eight organic solvents in vitro using pig skin PLoS ONE, 13(10,10), e0205458
Data Type:	Skin permeability
HERO ID:	5557704

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN (dichloromethane; CASRN 75-09-2).
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from a manufacturer (Merck). Although a lot/batch number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (>99.5%). The purity of the test substance was such that any observed effects are highly likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	The metric is not relevant to the study type. Guidelines for this study type suggest that data for relevant reference chemicals should be available (either by being tested concurrently or based on historical data). In this study, 38 organic solvents were tested; results from this study were compared to data from previous in vivo and in vitro data.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The metric is not relevant to the study type.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were provided in adequate detail (including source of skin, methods of preparation, storage conditions, and composition of the receptor fluid).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	The metric is not relevant to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Details regarding test substance storage and/or preparation were reported. The study indicates DCM (neat or diluted in water) was added to the donor compartment and capped with a glass stopper (presumed to be an alternative to using a charcoal filter for volatile substances as cited by guidelines for studies of this type). In addition, it was noted that the test substance remained soluble in the receptor fluid. The lack of additional details is not expected to substantially impact the study results.

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Study Citation: L. Schenk, M. Rauma, M. N. Fransson, G. Johanson (2018). Percutaneous absorption of thirty-eight organic solvents in vitro using pig skin PLoS ONE, 13(10,10), e0205458
Data Type: Skin permeability
HERO ID: 5557704

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of DCM exposure were reported; exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations (1% diluted in water and 100% "neat") were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure for the 38 chemicals tested ranged from 4 to 9 hours (not specified for individual chemicals).
	Metric 12:	Exposure Route and Method	High	× 1	1	Two concentrations of the test substance were utilized. The study authors indicated that neat chemicals and water dilutions were used because properties might vary significantly amongst these solutions.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The metric is not relevant to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test system was described in adequate detail. Pig skin was obtained from commercial breeders.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per group (n = 6) were adequate to address the outcome of interest (typically at least 4 replicates recommended for studies of this type).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology addressed the outcome of interest. The range of detection of chemicals in the receptor fluid suggested that the methods of assessment were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups (sampling of receptor fluid for GC analysis every 10 min for first 60 min, every 20 min for 60 min, then every 30 minutes).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	The metric is not relevant to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The metric is not relevant to the 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	Low	× 1	3	It was unclear whether freezing the skin samples and thawing affected baseline permeability.
Domain 7: Data Presentation and Analysis						

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Study Citation:	L. Schenk, M. Rauma, M. N. Fransson, G. Johanson (2018). Percutaneous absorption of thirty-eight organic solvents in vitro using pig skin PLoS ONE, 13(10,10), e0205458
Data Type:	Skin permeability
HERO ID:	5557704

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	Information with respect to steady-state flux and apparent permeability coefficient values calculations were described in the study report.
	Metric 23:	Data Interpretation	High	× 2	2	The study indicated that time course of test substance detected in the receptor medium would be used to determine the apparent permeability coefficient. The study described coefficients (relative terms) and that corresponded to moderate (10-4 cm/hr) to very high (10-2 cm/hour) permeabilities.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	The metric is not relevant to the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Only a summary of the data for DCM was provided in the study report. The study indicates that data are available online (could not access these data for for this review).
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 Subchronic (30-90 days)

Table 9: **Animal toxicity evaluation results of Kirschman et al 1986 for subchronic drinking water experiments in rats and mice study on hepatic, hematological and immune, adult exposure body weight, renal and clinical chemistry/biochemical outcomes**

Study Citation:	Kirschman, JC; Brown, NM; Coots, RH; Morgareidge, K (1986). Review of investigations of dichloromethane metabolism and subchronic oral toxicity as the basis for the design of chronic oral studies in rats and mice Food and Chemical Toxicology, 24(9), 943-949
Data Type:	Subchronic drinking water experiments in rats and mice
HERO ID:	730551

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified clearly by name with identified impurities and concentrations.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from a manufacturer.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Paper reports that specifications for the test substance to be used in a series of experiments include purity of >99.0%, but descriptions of the test material actually used in the subchronic rat and mouse experiments do not report purity. Food grade DCM was used in the 90-day study without further description. Yet, the study does state that the purity should be greater than that specified in the section discussing the test substance (> 99%). Thus, this omission is not likely to have an impact on the study results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The paper does not specify how the control group was treated, but as the study is a drinking water study it is reasonable to assume that the controls received water without test material.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required for this type of study
	Metric 6:	Randomized Allocation	Low	× 1	3	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	Study does not report methods for preparation or assessment of stability; these would be critically important for a drinking water study of DCM given its volatility. Although the preparation and storage was not described, the article notes that DCM was analyzed to estimate the doses. There could still be some significant impacts from volatilization depending on how often the authors analyzed DCM in water.

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Study Citation:	Kirschman, JC; Brown, NM; Coots, RH; Morgareidge, K (1986). Review of investigations of dichloromethane metabolism and subchronic oral toxicity as the basis for the design of chronic oral studies in rats and mice Food and Chemical Toxicology, 24(9), 943-949
Data Type:	Subchronic drinking water experiments in rats and mice
HERO ID:	730551

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration (e.g., ad lib or controlled) were not reported. Given that the authors analyzed for DCM and measured the consumption of water, the lack of details regarding consistency of exposure administration should not result in a result of 'unacceptable' for this study.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	The reported doses could not be verified. Study reports that analytical concentrations were used, but does not report these values or the method used to measure them. Water intake and body weight data were not reported, and decreased water consumption and body weights with higher DCM concentrations were noted in both species. Given that the authors analyzed for DCM and measured the consumption of water, the lack of details should not result in a result of 'unacceptable' for this study. Thus, the metric result was changed to 'low.'
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	The exposure frequency was not reported, but as a drinking water study is assumed to be 7 days per week. The exposure duration was reported and appropriate for the study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three dose groups plus control were tested. The overall dose range (high to low) was 10-fold and the spacing was typical for this type of study. The highest dose did result in some evidence of toxicity.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Drinking water administration appears to have been a poor choice given the observed decrease in water intake (potentially due to palatability) and potential for volatilization of DCM from the drinking water (study did not discuss stability of the test material). Authors did not describe any efforts to mitigate these issues.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Source, age, health status, and starting body weight were not reported for either species
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported for either species.

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Study Citation: Kirschman, JC; Brown, NM; Coots, RH; Morgareidge, K (1986). Review of investigations of dichloromethane metabolism and subchronic oral toxicity as the basis for the design of chronic oral studies in rats and mice Food and Chemical Toxicology, 24(9), 943-949
Data Type: Subchronic drinking water experiments in rats and mice
HERO ID: 730551

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported (20/sex/group for both rats and mice) and exceeded typical numbers and guideline recommendations for a study of this type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Methods for outcome assessment were incompletely reported (e.g., missing hematology and clinical chemistry parameters assessed, and missing list of organs weighed and/or examined microscopically)
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Study did not report how outcome assessment was executed across study groups
	Metric 18:	Sampling Adequacy	Low	× 1	3	Tabular results show adequacy of sampling for histopathology, but no information on sampling for clinical chemistry, hematology, or organ weights was provided.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Does not report blinding of assessors, but outcomes were not subjective. Although histopathology is subjective, conventional practice is that researchers are not blinded unless slides need to be evaluated a second time.
	Metric 20:	Negative Control Response	Low	× 1	3	inadequate information was available to assess suitability of the control response for any endpoint other than selected histopathology results.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Study reported decreased drinking water intake in both rats and mice with increasing dose. However, the authors analyzed for DCM and measured the consumption of water; therefore, the lack of details regarding consistency of exposure administration, although of concern, should not be a critical flaw.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	There were no health outcomes unrelated to exposure in rats, but in mice there were 6 deaths or moribund sacrifices (2 control, 2 low dose, and 2 mid-dose) unrelated to exposure. Although deaths occurred across doses in mice, they did not exceed 10%.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Kirschman, JC; Brown, NM; Coots, RH; Morgareidge, K (1986). Review of investigations of dichloromethane metabolism and subchronic oral toxicity as the basis for the design of chronic oral studies in rats and mice Food and Chemical Toxicology, 24(9), 943-949
Data Type:	Subchronic drinking water experiments in rats and mice
HERO ID:	730551

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	Statistical analysis was either not reported or not performed. Histopathology data are reported in sufficient detail to enable statistical analysis, but body weight, hematology, clinical chemistry, and organ weights were not reported quantitatively.
	Metric 24: Reporting of Data	Low	× 2	6	Body weight, hematology, clinical chemistry, and organ weights were not reported quantitatively but were described qualitatively. Histopathology results were reported quantitatively.
Overall Quality Determination‡		Low		2.5	
Extracted		No			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 10: **Animal toxicity evaluation results of General et al 1976 for a 90-day oral toxicity study in dogs study on mortality, body weight, neurological/behavioral, hematological and immune, ocular and sensory, clinical chemistry/biochemical, renal, hepatic, cardiovascular, endocrine, gastrointestinal, respiratory, skin and connective tissue, and thyroid outcomes**

Study Citation:	General Electric Company (1976). Dichloromethane ninety day oral toxicity study in dogs
Data Type:	90-d oral toxicity study in dogs
HERO ID:	4213649

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Dichloromethane identified by name and chemical structure and mol wt.
	Metric 2:	Test Substance Source	Medium	× 1	2	The compound was-received from the General Electric Company, Mount Vernon, Indiana on December 10, 1975. The compound was a clear liquid and was identified as "Dichloromethane* Reagent, A.C.S. CH2C12 FW 84.94 DX835 5509 Matheson Coleman & Bell Manufacturing Chemists". Note from study author: The above description is not totally accurate. The compound was furnished to IR&DC in containers labeled as indicated above but the actual contents were not from the indicated source. The contents were withdrawn on 12/4/75 from a purchased railroad tank -car of methylene chloride purchased from Dow Chemical certified to meet GE plastics Incoming Material Specification PCM-l-Sl. This methylene chloride is typical of that being used currently to produce Lexan® polycarbonate resin in the Mt. Vernon plant.
	Metric 3:	Test Substance Purity	Low	× 1	3	Not reported; study authors state "This methylene chloride is typical of that being used currently to produce Lexan® polycarbonate resin in the Mt. Vernon plant."
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent controls administered 13.33 ml of distilled water/kg-d on the same regimen as treated dogs.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for this type of study
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						

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Study Citation: General Electric Company (1976). Dichloromethane ninety day oral toxicity study in dogs
Data Type: 90-d oral toxicity study in dogs
HERO ID: 4213649

Domain		Metric	Rating†	MWF*	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The compound was dissolved in distilled water at a concentration of 15 mg/ml for gavage administration. Storage not reported (including methods to control volatilization).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volume differed between groups (13.33 ml/kg-d for 0 and 200 mg/kg-d; 3.33 ml/kg-d for 50 mg/kg-day; 0.83 ml/kg-d for 12.5 mg/kg-d). But likely to resulted in only minimal differences given that the vehicle is distilled water.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	0, 12.5, 50, or 200 mg/kg-d via gavage
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	90-d; it is assumed that dogs were dosed 7/days per week but this is not explicitly stated.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	3 exposure groups plus control; high-dose may not have been high enough (no exposure-related findings).
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Beagle dogs; 7.9-12.6 kg (male) or 5.4-11.3 kg (female)
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Consistent between groups. Individual housing in temperature and humidity controlled room. Water available ad libitum. 3000 g of food given per day. Temp and humidity not reported.
	Metric 15:	Number per Group	High	× 1	1	4/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	PECO: Hepatic - clinical chemistry, histo Neurological/Behavior - clinical signs, histo Other: Renal - clinical chemistry, urinalysis, histo Repro - histo Hematological or immunology - hemato, histo Gastrointestinal (histo) Respiratory (histo) Endocrine (histo) Musculoskeletal (histo) Cardiovascular (histo) Thyroid (histo) Ocular and Sensory (histo, ophthalmoscopy) Bd wt, mortality

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Study Citation: General Electric Company (1976). Dichloromethane ninety day oral toxicity study in dogs
Data Type: 90-d oral toxicity study in dogs
HERO ID: 4213649

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups; histology only assessed in control and high-dose (per protocol). Low- and mid-dose histology not evaluated due to lack of effects at high-dose.
	Metric 18:	Sampling Adequacy	High	× 1	1	4/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Study endpoints do not require blinding.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control reported; no deviations from standard reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting BW range reported. No exposure-related changes in BW or food consumption.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	No statistics reported by study authors. Data reporting adequate to perform independent statistics.
	Metric 24:	Reporting of Data	High	× 2	2	Comprehensive data tables.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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

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

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

Table 11: **Animal toxicity evaluation results of Dow 1961 for a 90-day dermal study in rabbits on mortality, body weight, neurological/behavioral, skin and connective tissue, hematological and immune, hepatic, renal, gastrointestinal, reproductive, thyroid, and cardiovascular outcomes**

Study Citation:	Dow Chem Co (1961). The results of chronic skin absorption studies on chlorothene and methylene chloride with cover letter
Data Type:	90-d dermal study - rabbits
HERO ID:	4213810

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Technical grade methylene chloride (chemical properties listed)
	Metric 2:	Test Substance Source	Low	× 1	3	Source of material not identified. No batch number or purity (identified as technical grade).
	Metric 3:	Test Substance Purity	Low	× 1	3	Reported as "technical grade"; % purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control was used
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive control (isopropyl alcohol) was used at 15, 100, and 500 mg/kg-d
	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	Lack of details re: preparation and storage may have an impact on results if the test substance was allowed to volatilize.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	In exposure groups, the total daily dose was divided into 4 equal parts that were administer directly onto the shaved skin of animals at 10 am, 12 pm, 2 pm, and 4 pm (5 days/week). Half of the animals had abraded skin (per group). At the end of exposure, the skin was wiped dry. Untreated controls were immobilized in a similar manner (no exposure).
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	0, 50, 100, 200, and 500 mg/kg-day (divided into 4 equal doses). In order to protect against accidental oral exposure, rabbits were restrained during exposure. In order to protect against accidental inhalation exposure, the stocks were situated in exhaust hoods leaving only the heads of the animals exposed to the external atmosphere. Loss of exposure to vaporization was not evaluated but animals were dosed 4 times/day (see metric 10), which would decrease evaporation.

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Study Citation: Dow Chem Co (1961). The results of chronic skin absorption studies on chlorothene and methylene chloride with cover letter
Data Type: 90-d dermal study - rabbits
HERO ID: 4213810

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	90 d, The total daily dose was divided into 4 equal parts that were administer directly onto the shaved skin of animals at 10 am, 12 pm, 2 pm, and 4 pm (5 days/week)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 dose groups plus control.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Dermal exposure under non-occluded conditions. Much smaller doses may have been administered due to vaporization of test material. Administering in 4 parts over 8 hrs may have decreased this, but occluded conditions should have been used.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	young adult male albino rabbits weighing 2-3 kg; source of animals not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Rabbits housed in cages with food available ad libitum (except during 8-hr exposure periods). No additional husbandry conditions reported.
	Metric 15:	Number per Group	Medium	× 1	2	4 males/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Daily mortality/clinical signs, weighed weekly; hematology assessed at 30, 60, 90 d; histology of skin, brain, heart, lung liver, kidney spleen, stomach, intestine and gonad and weight of brain, lung, heart, liver, stomach, kidney, spleen, gonad, and thyroid evaluated at 30 d (1/group) and 90 d (1/group). Other 2/group maintained for 30d observation.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups
	Metric 18:	Sampling Adequacy	Unacceptable	× 1	4	Organ weights and histology only assessed in 1/group at 30 and 90 days.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Examined endpoints did not require blinding.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No exposure-related changes.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						

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Study Citation:	Dow Chem Co (1961). The results of chronic skin absorption studies on chlorothene and methylene chloride with cover letter
Data Type:	90-d dermal study - rabbits
HERO ID:	4213810

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Unacceptable	× 1	4	No statistics reported. Body weights and hematology reported with adequate data for independent analysis, but of low power due to low animal number. Histological and organ weight data cannot be evaluated statistically (only 1/group per sacrifice).
	Metric 24: Reporting of Data	High	× 2	2	Detailed data tables.
Overall Quality Determination‡		Unacceptable⋆⋆		1.9	
Extracted		No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 12: **Animal toxicity evaluation results of Dow 1988 for a 13-week inhalation study on neurological/behavioral, ocular and sensory, and body weight outcomes**

Study Citation:	Dow Chemical Company (1988). Neurotoxicological examination of rats exposed to (DCM) vapor for 13-wks & evaluation of the acute neuropharmacologic effects of (DCM) in rats (final reports) (sanitized) w-letter
Data Type:	13-wk inhalation neurotox
HERO ID:	4213909

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Dichloromethane; physical properties reported.
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical Co (lot TA861111D). Identity confirmed by lab.
	Metric 3:	Test Substance Purity	High	× 1	1	99.95%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent controls were included. In addition, since at metabolic saturation (high dose), DCM is know to induce 10% COHb, an additional group of rats was exposed to 135 ppm CO to induce 10% COHb in the absence of DCM.
	Metric 5:	Positive Controls	Medium	× 1	2	Positive controls are sometimes used in neurobehavioral testing, but were not used in this study. Positive controls were not indicated for the majority of endpoints (e.g. histopathology, electrophysiology)
	Metric 6:	Randomized Allocation	Medium	× 1	2	Animals stratified by weight then randomly assigned (BW is nonrandom component.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Stability of substance confirmed (no changes before and after study). Detailed description of vapor generation. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Detailed description of vapor generation and monitoring of exposure (1-2 times/hr) in chambers.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target, nominal, and analytical exposure levels reported. Mean analytical (and nominal) concentrations of DCM present in the chambers during exposures were 50.0 (52.8), 200 (209), and 2000 (2127) ppm for the targeted levels of 50, 200, and 2000 ppm, respectively.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	13 wk, 6 hr/d, 5 d/wk

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Study Citation: Dow Chemical Company (1988). Neurotoxicological examination of rats exposed to (DCM) vapor for 13-wks & evaluation of the acute neuropharmacologic effects of (DCM) in rats (final reports) (sanitized) w-letter
Data Type: 13-wk inhalation neurotox
HERO ID: 4213909

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 exposure levels plus control. Exposure levels selected based on toxicokinetic properties: clearly below saturation (50 ppm), just below saturation (200 ppm), and well above saturation (2000 ppm). While no exposure-related effects were noted at highest exposures, higher exposure levels are not warranted (metabolic saturation).
	Metric 12:	Exposure Route and Method	High	× 1	1	Whole-body inhalation; dynamic chamber (4.1 m3) with airflow of 800 L/min (~12 air changes per hour)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	F344 rats, 16 wk old (Charles River). Check for health status upon arrival. Initial BW not available in report. Several tables (including Table 8 containing BW data) are missing - blank pages are labeled with "POOR COPY". Strain identified as having "general acceptance in neurotoxicity testing, availability of historical data and a reliable commercial supply.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housed one per cage, stainless steel cages with wire mesh floors; conditions approximately 22 degrees C, 50% humidity, and 12 hr light-dark cycle. Food and water available ad libitum except during exposure.
	Metric 15:	Number per Group	High	× 1	1	12/sex/group (plus 2 extra rats/sex to compensate for unplanned losses).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Comprehensive neurological testing (FOB, grip strength, flash evoked potentials, cortical flicker fusion, auditory brainstem responses, somatosensory evoked potentials, caudal nerve action potentials, detailed histopathology of nervous tissues). Body weights and body and tail temperatures also recorded.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	8-12/group evaluated per functional test; 6/group sacrificed for histology after behavioral testing. Remaining 6/group were help post-exposure and "eventually" submitted to necropsy. Only control and high dose tissues were examined for histology; low- and mid-dose not examined due to lack of findings at the high dose (per protocol). It doesn't appear that post-exposure group tissues were examined.

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Study Citation:	Dow Chemical Company (1988). Neurotoxicological examination of rats exposed to (DCM) vapor for 13-wks & evaluation of the acute neuropharmacologic effects of (DCM) in rats (final reports) (sanitized) w-letter
Data Type:	13-wk inhalation neurotox
HERO ID:	4213909

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	High	× 1	1	For subjective evaluations (e.g. FOB), assessors were blinded.
	Metric 20:	Negative Control Response	High	× 1	1	Controls responses reported. There were some issues with findings in the flash evoked potential test in controls and exposure groups; this is discussed in the confounders section below and not rated here.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial BW not available; body weights were comparable to control at end of study. Respiratory rate was not evaluated; unknown if bradypnea occurred.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Findings unrelated to treatment: 1. Persistent muscular weakness in one hind leg in all groups due to injection of ketamine and xylazine (anesthesia for cranial implant surgery) was recognized during weekly clinical exams and FOB. This is not expected to impact results. 2. Male rats housed in the top-tier of the cage rack were inadvertently exposed to overhead florescent lighting without the standard translucent plastic cover (was accidently dislodged). Rats effected included 4 male rats/group. This impacted results in the flash evoked potential test; therefore, results from these rats were excluded from analysis. Therefore, it did not impact outcome assessment.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Detailed description of statistics reported.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data is referred to for body weight and several neurological tests, but several tables are missing from the report (blank page with POOR COPY written on it); however, none of the findings were significant. Findings that were near-significant (FEP) are quantitatively reported. Gross and microscopic pathology data reported quantitatively.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	Dow Chemical Company (1988). Neurotoxicological examination of rats exposed to (DCM) vapor for 13-wks & evaluation of the acute neuropharmacologic effects of (DCM) in rats (final reports) (sanitized) w-letter
Data Type:	13-wk inhalation neurotox
HERO ID:	4213909

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

4 Chronic (>90 days)

Table 13: **Animal toxicity evaluation results of Serota et al 1986 for a 2-year oral cancer bioassay in rats study on cancer, reproductive, hematological and immune, neurological/behavioral, renal, hepatic, ocular and sensory, cardiovascular, clinical chemistry/biochemical, endocrine, gastrointestinal, mortality, musculoskeletal/motor function, body weight, respiratory, skin and connective tissue, thyroid, and mortality outcomes**

Study Citation:	Serota, D. G., Thakur, A. K., Ulland, B. M., Kirschman, J. C., Brown, N. M., Coots, R. H.,Morgareidge, K. (1986). A two-year drinking-water study of dichloromethane in rodents: I. Rats Food and Chemical Toxicology, 24(9), 951-958
Data Type:	2-year oral cancer bioassay in rats
HERO ID:	730592

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	food grade dichloromethane
	Metric 2:	Test Substance Source	Medium	× 1	2	Diamond Shamrock Industries, with certificate of analysis. Batch no. not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	"Food grade" - percent purity not reported. Analysis at 32, 52, 78 and 104 wk of study confirmed that the DCM sample was stable throughout the study period. A previous study (Kirschman, 1986) was consulted, which has purity information, .
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Two untreated control groups were run concurrently (deionized water only).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not necessary for study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Rats were randomly allocated into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Detailed descriptions of storage and preparation of test substance with periodic testing for stability and accuracy of dosing solutions.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent between groups. Regular testing of water for consistency of exposure solutions.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The actual DCM intakes were determined by study authors from measured DCM concentrations in the drinking-water and the actual body weights and water consumption values. Target: 5, 50, 125, 250,and 250(recovery group) mg/kg-d. Measured: 6, 52, 125, 235, and 232 mg/kg-d, respectively (males); 6, 58, 136, 263, and 269 mg/kg-d, respectively (females).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	104 wks in main study; 78 wk plus 26-wk recovery in recovery group.

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Study Citation:	Serota, D. G., Thakur, A. K., Ulland, B. M., Kirschman, J. C., Brown, N. M., Coots, R. H.,Morgareidge, K. (1986). A two-year drinking-water study of dichloromethane in rodents: I. Rats Food and Chemical Toxicology, 24(9), 951-958
Data Type:	2-year oral cancer bioassay in rats
HERO ID:	730592

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	4 doses plus control. Dose levels were selected on the basis of findings from subchronic and pharmacokinetic studies of DCM.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Drinking water. There is no discussion of volatility but paper does report that the concentrations were analyzed and demonstrated that they were stable.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	F344 rats (Charles River Breeding Laboratory); ~7 wk old at study initiation. Starting body weight was not reported. Health status is not explicitly stated.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Consistent between groups. Detailed reporting of husbandry conditions.
	Metric 15:	Number per Group	High	× 1	1	85/sex/group in exposure groups and control group 1 in main study (35/sex/group slated for interim sacrifices, 50/sex/group for terminal sacrifices); 50/sex/group in control group 2; 25/sex/group in recovery group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Monitored mortality, clinical signs, body weight, and food/water consumption throughout the study. Comprehensive histopathology, organ weights, hematology, serum chemistry, urinalysis. Ophthalmological evaluation.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Outcome evaluated for all animals, which is adequate for this study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported; unexpected findings were not reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW not reported; small but statistically significant decreases in BW and water consumption were qualitatively reported for >=125 mg/kg-day groups. Concomitant decreased in food consumption noted for first 13 wks. Based on designation of "small", these are not expected to impact results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No infections reported. Mortality rates similar, and similar incidental and age-related lesions in all groups (except liver).

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Study Citation:	Serota, D. G., Thakur, A. K., Ulland, B. M., Kirschman, J. C., Brown, N. M., Coots, R. H.,Morgareidge, K. (1986). A two-year drinking-water study of dichloromethane in rodents: I. Rats Food and Chemical Toxicology, 24(9), 951-958
Data Type:	2-year oral cancer bioassay in rats
HERO ID:	730592

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Detailed description of various statistical tests used. Tumor analysis included unadjusted and adjusted for intercurrent mortality.
	Metric 24: Reporting of Data	Medium	× 2	4	Hepatic nonneoplastic and neoplastic lesions reported quantitatively. Statistically significant changes in body weight, food consumption, drinking water intake, hematology, and clinical chemistry were reported qualitatively. Organ weight findings were considered unrelated to treatment despite occasional dose-dependent findings (reported qualitatively). The remaining results were reported qualitatively (lack of compound-related effects).
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 14: **Animal toxicity evaluation results of NTP 1986 for a 2-year inhalation cancer bioassay study on cancer outcomes**

Study Citation:	NTP (1986). NTP toxicology and carcinogenesis studies of dichloromethane (methylene chloride) (CAS No. 75-09-2) in F344/N rats and B6C3F1 mice (inhalation studies) 306 1-208
Data Type:	2-year inhalation cancer bioassay
HERO ID:	732410

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Name, physiochemical properties, structure, and CASRN were reported.
	Metric 2:	Test Substance Source	High	× 1	1	Source, lot numbers, and data from identity analyses were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control animals were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control animals were not required
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The equipment and method used to generate the test substance concentrations were recorded.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations reported for 2-year study, and the method used for measuring concentration was reported and appropriate .
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration and frequency were reported and appropriate for a cancer bioassay.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Exposure groups and concentration spacing were adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The test substance was heated in duct before entering chambers; air concentrations continually measured - concentrations are within 90-110% for the majority of time.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics were reported. Health status was assessed but not reported. High level of mononuclear cell leukemia in all male rats but incidence in controls is similar to historical controls.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and were adequate.

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Study Citation:	NTP (1986). NTP toxicology and carcinogenesis studies of dichloromethane (methylene chloride) (CAS No. 75-09-2) in F344/N rats and B6C3F1 mice (inhalation studies) 306 1-208
Data Type:	2-year inhalation cancer bioassay
HERO ID:	732410

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 outcome(s) of interest and was sensitive.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported.
	Metric 19:	Blinding of Assessors	High	× 1	1	Coded slides were re-evaluated by the Pathology Working Group when the original and quality assurance pathologists disagreed. and was conducted in a 'blinded' fashion.
	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	DCM is a respiratory irritant but respiratory rate measurement was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	An unusually high incidence of mononuclear cell leukemia was seen (all male concentrations and in controls). This is expected to have some impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 15: **Animal toxicity evaluation results of Burek et al 1984 for 2-year cancer bioassay study on cancer, hepatic, and renal outcomes**

Study Citation:	Burek, JD; Nitschke, KD; Bell, TJ; Wackerle, DL; Childs, RC; Beyer, JE; Dittenber, DA; Rampy, LW; McKenna, MJ (1984). Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters Fundamental and Applied Toxicology, 4(1), 30-47
Data Type:	2-year cancer bioassay
HERO ID:	29091

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 chemical formula.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was not given; however, analytical verification was accomplished by GC. Manufacturer and lot numbers were given in the unpublished OxyChem (1992) report (4214046).
	Metric 3:	Test Substance Purity	High	× 1	1	Described as technical grade, but analysis by GC indicated purity >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required for this type of study
	Metric 6:	Randomized Allocation	High	× 1	1	Computerized randomization procedure.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The equipment and method for vapor generation are not well described; however, there was close agreement between daily nominal and analytical values. The method for vapor generation was described by the unpublished report (OxyChem, 1992; 4214046).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Range of analytical concentration did not deviate more than 10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 hours/day, 5 days/week, 2-year duration
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Dose response relationships were evident, but unclear if lowest dose was low enough (i.e., liver histopath. changes. at all doses).
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	~95 animals/sex/group

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Study Citation:	Burek, JD; Nitschke, KD; Bell, TJ; Wackerle, DL; Childs, RC; Beyer, JE; Dittenber, DA; Rampy, LW; McKenna, MJ (1984). Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters Fundamental and Applied Toxicology, 4(1), 30-47
Data Type:	2-year cancer bioassay
HERO ID:	29091

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed similarly across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	All dose groups were evaluated for all parameters.Due to deaths in pre-assigned animals to be sampled for various outcomes, different numbers of animals were sometimes taken for sampling.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No reference to blinding was made, but all measures were objective. Although histopathology evaluation is not objective, the first evaluation is not traditionally blinded but if additional evaluation of histopathology is needed, reviewers are blinded.
	Metric 20:	Negative Control Response	Low	× 1	3	Elevated incidence of histopathology lesions in controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported; test substance is a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Rats had a common viral infection early in the treatment period; salivary gland tumor results may be confounded by this infection. Endpoints other than salivary gland tumors may also be affected.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	Medium	× 2	4	The data for many outcomes was reported in text. Only selected finding were reported for histopathology. A medium rating is given because data tables are provided in the unpublished study report (OxyChem, 1992; 4214046).
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 16: **Animal toxicity evaluation results of Aiso et al 2014 for a 2 year cancer bioassay in rats and mice**

Study Citation:	Aiso, S; Take, M; Kasai, T; Senoh, H; Umeda, Y; Matsumoto, M; Fukushima, S (2014). Inhalation carcinogenicity of dichloromethane in rats and mice Inhalation Toxicology, 26(8,8), 435-451
Data Type:	2 year cancer bioassay in rats and mice
HERO ID:	4238148

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Study authors identified the chemical definitely and provided CAS number.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source reported, batch/lot number not provided, but each lot of the test substance was analyzed by analytical methods for its purity and stability.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported to be > 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent control group exposed to clean air was handled in same manner as test chemical-exposure treated groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not typical for this type of study.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Animals were allocated by stratified randomization procedure into body-weight matched test and control groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance stored in air tight bottles at room temperature and analyzed for stability; no decomposition products or impurities detected. Vapor generated by bubbling clean air through liquid test substance and diluting to desired concentrations.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups. This included exposure chamber descriptions, time of day of exposures, methods for atmosphere generation, and methods for analyzing chamber concentrations etc.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and mean (SD) analytical concentrations were reported and SDs and within acceptable range of deviation (SDs were <1% of mean). Concentrations in the chambers monitored at 15 min intervals by GC.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The study authors reported exposure frequency and duration of exposure appropriate for this study type and/or outcome(s) of interest.

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Study Citation: Aiso, S; Take, M; Kasai, T; Senoh, H; Umeda, Y; Matsumoto, M; Fukushima, S (2014). Inhalation carcinogenicity of dichloromethane in rats and mice Inhalation Toxicology, 26(8,8), 435-451
Data Type: 2 year cancer bioassay in rats and mice
HERO ID: 4238148

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Exposure concentrations selected based on subchronic study conducted by the same laboratory. The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure were reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The study authors reported species, strain, sex, health status, age, and starting body weight of the test animals. Test animals were obtained from a commercial source and the animal strain was appropriate for the evaluation of carcinogenesis.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Study authors reported all husbandry conditions for the animals including temperature, humidity, and light-dark cycle.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported which was appropriate for a 2-year cancer study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology reported. The study was conducted in accordance with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Study authors provided details of outcome assessment protocol; no inconsistencies were reported.
	Metric 18:	Sampling Adequacy	High	× 1	1	Except for testicular neoplasms in one male control animal, 1 or 2 male or female animals for thyroid tumors all the animals were evaluated for tumors. However, this is unlikely to impact the interpretation of the data.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses for the negative controls were reported and were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There was no significant difference in the initial body weight, food or water intake between any study groups of either sex and their respective controls. Although DCM is a potential respiratory irritant, the authors did not report the respiratory rate.

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Study Citation:	Aiso, S; Take, M; Kasai, T; Senoh, H; Umeda, Y; Matsumoto, M; Fukushima, S (2014). Inhalation carcinogenicity of dichloromethane in rats and mice Inhalation Toxicology, 26(8,8), 435-451
Data Type:	2 year cancer bioassay in rats and mice
HERO ID:	4238148

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Health Outcomes Unrelated to Exposure	High	× 1	1	Authors reported details of animal attrition and health outcomes and did not observe any health effects unrelated to exposure.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Authors clearly described the statistical methods which were appropriate for the dataset analysis.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex, and negative findings were reported qualitatively or quantitatively.
Overall Quality Determination‡		High		1.1	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 17: **Animal toxicity evaluation results of Hazleton et al 1983 for 2-year oral cancer bioassay study on cancer and hepatic outcomes**

Study Citation:	Hazleton Laboratories (1983). 24-month oncogenicity study of methylene chloride in mice: Final report					
Data Type:	2-year oral cancer bioassay					
HERO ID:	29131					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Identified by name. CASRN and structure not provided.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot no. provided.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity analyses were conducted every 6 months, but results were reported in an appendix that was NOT included in the pdf.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	2 drinking water control groups
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not required for this type of study.
	Metric 6:	Randomized Allocation	High	× 1	1	Computerized randomization process.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well described. Pilot study examined stability and homogeneity of test substance in drinking water.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were calculated by study authors from analytical measurement of dw concentrations, measured intake and bw values.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	24 months is appropriate for cancer bioassay; frequency was not explicitly reported, but 7 days/week is assumed based on reference to observation conducted on Saturday and Sunday.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Narrow spacing between doses (nominal doses of 0, 60, 125, 185, 250 mg/kg-bw/day); no clear dose-response across groups.
	Metric 12:	Exposure Route and Method	High	× 1	1	Drinking water concentrations were measured analytically.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Commonly used mouse strain, obtained from commercial source.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were well-reported and adequate.

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Study Citation: Hazleton Laboratories (1983). 24-month oncogenicity study of methylene chloride in mice: Final report
Data Type: 2-year oral cancer bioassay
HERO ID: 29131

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	High	× 1	1	>50/group and some had 50/group
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Low	× 2	6	Hematology data were limited to leukocyte count and differential, no clinical chemistry data, no organ weight data.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	
	Metric 18: Sampling Adequacy	High	× 1	1	Outcome evaluated for all animals
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported (initial histopath).By convention, initial histopathological exams not typically blinded.
	Metric 20: Negative Control Response	Low	× 1	3	Elevated incidence of liver histopath. lesions in controls. Also, convulsions seen in all groups without identified cause.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Reported decrease in wate3r consumption in high dose males; however, the pdf does not contain the data tables and the magnitude of the decrease is not reported. Authors calculated actual doses (mg/kg-bw/day) so impact of lower water consumption on results should be minor.
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	Convulsions were reported in controls and treated mice. Without an explanation as to cause, it is not clear how the convulsions (or the cause of the convulsions) may have confounded results.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were well-described and appropriate.
	Metric 24: Reporting of Data	Low	× 2	6	Data tables are missing from the pdf. Results are described in text.
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 =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

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

Table 18: **Animal toxicity evaluation results of Maltoni et al 1988 for an oral and inhalation cancer bioassay study on cancer outcomes in rats and mice**

Study Citation:	Maltoni, C; Cotti, G; Perino, G (1988). Long-term carcinogenicity bioassays on methylene chloride administered by ingestions to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats Annals of the New York Academy of Sciences, 534 352-366
Data Type:	Cancer-rat, mice oral, rat inhalation
HERO ID:	29235

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, structure, molecular formula and weight.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source was identified, but additional details were not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity and composition were reported and such that effects were likely due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were included in all experiments but unclear if inhalation controls were exposed to air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric not applicable for this study.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Oral: doses administered in olive oil, but preparation and storage conditions were not reported; lack of Inhalation: atmosphere generations methods were not reported but concentrations were monitored. It is not known whether the method of preparation and storage might have contributed to volatilization.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Oral: appear to be consistent; Inhalation: unclear, as no details were provided
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses and concentrations reported for all experiments.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Data reported but rationale not provided for changes in the inhalation study.

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Study Citation:	Maltoni, C; Cotti, G; Perino, G (1988). Long-term carcinogenicity bioassays on methylene chloride administered by ingestions to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats Annals of the New York Academy of Sciences, 534 352-366
Data Type:	Cancer-rat, mice oral, rat inhalation
HERO ID:	29235

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Ingestion: Number of exposure groups and spacing (2 groups) were adequate for the purposes of the study. The inhalation study is unacceptable for our purposes because it is not relevant (it doesn't meet the PECO). We are not evaluating studies with 1 concentration and there is only one concentration group for adults and only one concentration group for offspring (embryos). Furthermore, these two concentrations differ.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure routes were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, and age were reported. Initial body weight and source were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Specifics regarding husbandry were not reported and could not be evaluated.
	Metric 15:	Number per Group	High	× 1	1	The numbers of animals for each study were appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The article is unclear regarding how long animals continued to be followed after the exposure was stopped at 64 weeks. Also, cancer studies are typically conducted for the lifetime of the rodents; because this study was stopped earlier, the sensitivity to measure the outcomes of interest is limited.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assess consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	The responses appeared to be adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Several parameters were not reported or appeared not to have been measured. DCM is a respiratory irritant and respiratory rate measurement was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	For both study types, no data on attrition or health outcomes were reported, but from the data reported, there does not appear to be health effects unrelated to treatment.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Maltoni, C; Cotti, G; Perino, G (1988). Long-term carcinogenicity bioassays on methylene chloride administered by ingestions to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats Annals of the New York Academy of Sciences, 534 352-366
Data Type:	Cancer-rat, mice oral, rat inhalation
HERO ID:	29235

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analyses were conducted, but were not described; however, sufficient data were present to conduct analysis for outcomes.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for tumor outcomes were reported in text and tables. Survival was discussed but quantitative values per dose are not reported, even in the text. It is difficult to interpret the tumor data without details regarding survival.
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 19: **Animal toxicity evaluation results of Nitschke et al 1988 for 2-year inhalation cancer bioassay study on cancer, mortality, clinical chemistry/biochemical, hematological and immune, respiratory, cardiovascular, gastrointestinal, ocular and sensory, musculoskeletal/motor function, endocrine, hepatic, reproductive, neurological/behavior, skin and connective tissue, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	Nitschke, KD; Burek, JD; Bell TJ; Kociba, RJ; Rampy, LW; McKenna, MJ (1988). Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats Fundamental and Applied Toxicology, 11(1), 48-59
Data Type:	2-year cancer bioassay
HERO ID:	29244

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and the lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity (reported as at least 99.5%, as determined by periodic gas chromatography analysis) was such that any observed effects were highly likely to be due to the test substance itself.
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 controls are not required for this type of study.
	Metric 6:	Randomized Allocation	High	× 1	1	The animals were randomly assigned to groups using a computer-derived randomization process.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported and methods were appropriate. Storage conditions were not reported; however, the test substance was periodically evaluated by gas chromatography and there was no indication of decomposition during the study.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Due to a lack of chambers of comparable size, the control animals remained in the animal holding room during each exposure period.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytically determined concentrations, based on the mean of daily time-weighted average concentrations, were reported for each group. The methods used to measure the chamber test substance (infrared spectroscopy, 1-2 times/hour) were reported and appropriate.

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Study Citation: Nitschke, KD; Burek, JD; Bell TJ; Kociba, RJ; Rampy, LW; McKenna, MJ (1988). Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats Fundamental and Applied Toxicology, 11(1), 48-59
Data Type: 2-year cancer bioassay
HERO ID: 29244

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and the outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified by the study authors (based on a previous study reporting no NOAEL [Burek et al. 1984] and using concentrations below, above, and intermediate to that resulting in saturation of the mixed function oxidase metabolism of DCM, as discussed on p. 49).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance. The number of air changes per hour was adequate (12/hour).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Starting body weight and health status at the beginning of the study were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions (temperature, humidity, light-dark cycle) were consistent.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported and appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes.
	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 outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No evaluations that were considered subjective were conducted and histopathological evaluations were not described as re-evaluation so I considered this metric N/A.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported; however, DCM is a potential respiratory irritant but respiratory rate measurement was not reported.

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Study Citation:	Nitschke, KD; Burek, JD; Bell TJ; Kociba, RJ; Rampy, LW; McKenna, MJ (1988). Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats Fundamental and Applied Toxicology, 11(1), 48-59
Data Type:	2-year cancer bioassay
HERO ID:	29244

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for datasets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were shown for each 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

5 Genetic toxicity studies

Table 20: **Animal toxicity evaluation results of Kramers et al 1991 for inhalation study on genetic mutations in Drosophila**

Study Citation:	P. G. N. Kramers, H. C. A. Mout, B. Bissumbhar, C. R. Mulder (1991). Inhalation exposure in Drosophila mutagenesis assays: Experiments with aliphatic halogenated hydrocarbons, with emphasis on the genetic activity profile of 1,2-dichloroethane Mutation Research, 252(1,1), 17-33
Data Type:	Mutations in Drosophila
HERO ID:	13933

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	For most experiments, a manufacturer and lot number was provided. For the short-term sex-linked recessive lethal (SLRL) experiment the test substance was obtained from a university without analytical verification (unacceptable).
	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	Negative control was indicated as 0 mg/m3 in air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	It is not clear whether positive controls are strictly required. MMS results were discussed, but it is unclear whether these were conducted concurrently. Historical controls are shown in Table 5, but no details on these controls were provided.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable to the study type. Randomization may not be necessary for Drosophila.
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	Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Measured concentrations were reported; however, the concentrations were not within +/- 10% (range > 20%; see footnotes to Table 1).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Several exposure times were used for SLRL mutations (6h, 1 and 2 weeks).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The study utilized 3 concentrations plus a control. Concentrations approaching those producing anesthesia were used as a practical limit.

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Study Citation:	P. G. N. Kramers, H. C. A. Mout, B. Bissumbhar, C. R. Mulder (1991). Inhalation exposure in Drosophila mutagenesis assays: Experiments with aliphatic halogenated hydrocarbons, with emphasis on the genetic activity profile of 1,2-dichloroethane Mutation Research, 252(1,1), 17-33
Data Type:	Mutations in Drosophila
HERO ID:	13933

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex and lifestage were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Temperature was reported (no other conditions were reported).
	Metric 15:	Number per Group	Not Rated	NA	NA	A publication was cited for the methodology details.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and is sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses appear appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	No confounding variables were assessed. These factors (e.g., body weights) are not expected to be applicable to the study type (i.e., a study in Drosophila).
	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	Tables cited in a different paper were used for statistical calculations.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	P. G. N. Kramers, H. C. A. Mout, B. Bissumbhar, C. R. Mulder (1991). Inhalation exposure in Drosophila mutagenesis assays: Experiments with aliphatic halogenated hydrocarbons, with emphasis on the genetic activity profile of 1,2-dichloroethane Mutation Research, 252(1,1), 17-33
Data Type:	Mutations in Drosophila
HERO ID:	13933

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 21: **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 DCM
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 methylene chloride (CH2Cl2).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
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	High	× 1	1	The number of exposure groups and dose spacing was 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, sex, age, and commercial source of the test animals were reported. Starting body weights of the test animals were not reported.
	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.

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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 DCM
HERO ID:	195230

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 these endpoints (n = 8 for low- and mid-dose; n = 15 for high-dose; n = 22 for vehicle controls).
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	It was reported that preliminary lethality studies showed death of 7/10 rats after 3825 mg/kg DCM, but this was not a dose in the current study (highest dose 1275 mg/kg DCM).
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‡			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 =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

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

Table 22: **Animal toxicity evaluation results of Mirsalis et al 1989 for unscheduled DNA synthesis in vivo**

Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for DCM
HERO ID:	200781

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as dichloromethane.
	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	Two concurrent solvent control groups were included (water and corn oil gavage) for rats. However, DCM was administered via intraperitoneal injection in saline. No matched controls were included for this route of exposure.
	Metric 5:	Positive Controls	High	× 1	1	Dimethylnitrosamine and 2-acetylaminofluorene were included as positive controls. Positive responses were observed from positive controls.
	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	High	× 1	1	The number of exposure groups and dose spacing was appropriate.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure were appropriate for the test substance; however, no rationale was provided for administering DCM by injection rather than gavage, as the other 23 chemicals were.
Domain 4: Test Organism						

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Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for DCM
HERO ID:	200781

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. Age of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 3 for all DCM-treated groups; n = 2 for corn oil controls at 2 hr; n = 52 for corn oil controls at 12 hours; n = 31 for water controls at 2 hours).
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	High	× 1	1	Fifty cells per slide and 3 slides per animal were assessed.
	Metric 19:	Blinding of Assessors	High	× 1	1	The slides were coded prior to analysis.
	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	High	× 2	2	Starting body weight ranges were 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 health effects unrelated to the test substance administration were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	No statistical analysis was performed on the data. A positive result was defined as an average net nuclear grain count exceeding 0, which was reported to be in line with the lab's historical controls (negative controls never exceeding an average net nuclear grain count of 0). These criteria are appropriate for the outcome of interest. Statistical analysis could be conducted based on the summary data (means, SEM, and n) provided in Table I.
	Metric 24:	Reporting of Data	High	× 2	2	The data were reported adequately.

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Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for DCM
HERO ID:	200781

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 23: **Animal toxicity evaluation results of Casanova et al 1992 for DNA-protein crosslinks and DNA binding in vivo**

Study Citation:	M. Casanova, D. F. Deyo, H. Heck (1992). Dichloromethane (methylene chloride): metabolism to formaldehyde and formation of DNA-protein cross-links in B6C3F1 mice and Syrian golden hamsters Toxicology and Applied Pharmacology, 114(1,1), 162-165
Data Type:	DNA-protein crosslinks and DNA binding in vivo for DCM
HERO ID:	730496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as dichloromethane (DCM) and [14C]dichloromethane ([14C]DCM).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial sources of the test substances were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The radiochemical purity of the [14C]DCM was reported to be 99%. The purity of the unlabeled DCM was reported to be 99.9%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	It appears that no negative controls were included in the study design. This is considered acceptable based on the study design (radiolabeled DNA binding).
	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	The preparation, handling, and storage of the test substance was described in detail and appropriate considering the volatility of the test substance.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	This metric is not applicable, as there was only one experimental condition (treatment group) per species.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	The animals were exposed to a constant concentration of 4006 ± 60 ppm unlabeled DCM for 2 days (6 hr/day). On the third day, the concentration of [14C]DCM decreased throughout the day on the third day from 4500 ± 250 ppm to 2500 ± 250 ppm. No rationale is provided for variable concentration of labeled on the third day. It is unclear whether the concentration on the third day decreased in a linear fashion. Therefore, it is not clear what an equivalent time-weighted average for the third day or for the 3-day exposure period is.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this endpoint.

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Study Citation:	M. Casanova, D. F. Deyo, H. Heck (1992). Dichloromethane (methylene chloride): metabolism to formaldehyde and formation of DNA-protein cross-links in B6C3F1 mice and Syrian golden hamsters Toxicology and Applied Pharmacology, 114(1,1), 162-165
Data Type:	DNA-protein crosslinks and DNA binding in vivo for DCM
HERO ID:	730496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	A single exposure group was used..
	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	High	× 2	2	The species, strain, sex, commercial source, age, and starting body weight range of the test animals were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Limited details about husbandry conditions were reported, but they appeared to be appropriate.
	Metric 15:	Number per Group	Low	× 1	3	For each experiment, n = 3 mice and n = 1 hamster. Each experiment was repeated 4 times. The number of hamsters per experiment is considered inadequate, though not unacceptable due to the repetition of the experiment (acknowledging that the exposures were likely slightly different between experiments).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for these endpoints.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	This metric is not applicable to the study design, as only one experimental condition (treatment group) per species was included.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It is unclear how many technical replicates were included in the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This metric is not applicable to the study design, as no negative controls were included.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting body weight ranges were 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 health effects unrelated to the test substance administration were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	No statistical analysis was performed on the data, as no negative controls were included in the study design.

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Study Citation:	M. Casanova, D. F. Deyo, H. Heck (1992). Dichloromethane (methylene chloride): metabolism to formaldehyde and formation of DNA-protein cross-links in B6C3F1 mice and Syrian golden hamsters Toxicology and Applied Pharmacology, 114(1,1), 162-165
Data Type:	DNA-protein crosslinks and DNA binding in vivo for DCM
HERO ID:	730496

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	The data were reported adequately.
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 24: **Animal toxicity evaluation results of Devereux et al 1993 for tumor analysis of Ras mutation in mice**

Study Citation:	T. R. Devereux, J. F. Foley, R. R. Maronpot, F. Kari, M. W. Anderson (1993). Ras proto-oncogene activation in liver and lung tumors from B6C3F1 mice exposed chronically to methylene chloride Carcinogenesis, 14(5,5), 795-801
Data Type:	tumor analysis of Ras mutation in mice
HERO ID:	730508

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Tests substance was identitified by name
	Metric 2:	Test Substance Source	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 3:	Test Substance Purity	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Control animals were included it is unclear if they were vehicle or untreated
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 9:	Reporting of Doses/Concentrations	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for the study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Single dose group was reported and was justified by study authors.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
Domain 4: Test Organism						

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Study Citation:	T. R. Devereux, J. F. Foley, R. R. Maronpot, F. Kari, M. W. Anderson (1993). Ras proto-oncogene activation in liver and lung tumors from B6C3F1 mice exposed chronically to methylene chloride Carcinogenesis, 14(5,5), 795-801
Data Type:	tumor analysis of Ras mutation in mice
HERO ID:	730508

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Animal characteristics were partially reported including species strain and sex but some details were missing. Animals are routinely used for the outome of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the outcome.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was inferred to be consistent 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	Not Rated	NA	NA	This metric is not applicable for the study type.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	Tumors were obtained from a cited bioassay (Kari et al., 1993). Minimal details regarding this bioassay were provided.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were not described but data reported was sufficient for independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data was reported for all outcomes and groups.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	T. R. Devereux, J. F. Foley, R. R. Maronpot, F. Kari, M. W. Anderson (1993). Ras proto-oncogene activation in liver and lung tumors from B6C3F1 mice exposed chronically to methylene chloride Carcinogenesis, 14(5,5), 795-801
Data Type:	tumor analysis of Ras mutation in mice
HERO ID:	730508

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: **Animal toxicity evaluation results of Graves et al 1994 for hepatic DNA damage in mice and rats**

Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA damage in animals exposed to DCM (rats and mice)
HERO ID:	730537

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	Manufacturer was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported as HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used (Fig. 4), but details were not described.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to 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	Not Rated	NA	NA	Inhalation exposure methods were cited to another publication (Green et al., 1988).
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Inhalation exposure methods were cited to another publication (Green et al., 1988).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in ppm and monitored analytically by GC. Concentrations did not vary widely (indicated as <+/-10% for 4000 ppm).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single-day exposure is appropriate for the outcome of interest. 3h and 6h durations were compared.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two exposure groups and a negative control were used (quantitative data were only provided for the highest concentration).
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Inhalation exposure methods were cited to another publication (Green et al., 1988).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, source and weight were reported and the species is routinely used for the outcome of interest. Age and health status were 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	Medium	× 1	2	n=3-4 per group, which is lower than recommended by the test guideline (n=5)
Domain 5: Outcome Assessment						

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Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA damage in animals exposed to DCM (rats and mice)
HERO ID:	730537

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment was inferred to be consistent across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses appeared to be adequate for the outcome.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported and may influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No difference in health outcomes among the study groups were reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were not reported but data was provided in graphical form with SD bars that may be interpreted for an independent analysis, however the N and error would be variable.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for the 2000 ppm group (3 or 6h) and 4000 ppm (3h) were not provided (general conclusions were provided in text).
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 26: **Animal toxicity evaluation results of Graves et al 1995 for DNA damage in vivo**

Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926					
Data Type:	DNA damage in vivo					
HERO ID:	730538					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as methylene chloride (MC).
	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, this is not expected to substantially impact the results given the short-term nature of the experiments.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were included (air-exposed).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. Although a positive control substance was not used, the study authors showed that a positive result could be induced in each tissue type (liver and lungs).
	Metric 6:	Randomized Allocation	Low	× 1	3	The allocation of animals to study groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Methods and equipment used to generate the test substance for inhalation experiments were cited to a previous publication.
	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	Low	× 2	6	Target concentrations were reported without ambiguity (in ppm). Concentrations were monitored continuously using gas chromatography. Although the analytical method is reliable, actual/measured concentrations were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency/duration were reported and appropriate for this study type. Similar studies expose animals for at least two days. In this study, exposures occurred over 1 to 5 days. Acute studies (single exposures) were considered acceptable because the test substance gave a positive response.

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Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926
Data Type:	DNA damage in vivo
HERO ID:	730538

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were considered adequate to address the purpose of the study (i.e., 4 exposure groups plus control for mouse studies).
	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, commercial source, and starting body weight range of the test animals were reported. The age of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported. Methylene chloride inhalation experiments were cited to another publication.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per exposure group (2 to 4 males) was lower than the number typically used for similar studies (i.e., at least 5 animals). However, the number of animals used in the study is unlikely to substantially impact the results. It is noted that data from Figure 2 represent one animal per time point (used to determine the time course of recovery).
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	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (2 replicates 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	No confounding variables were identified. Data for respiratory rates were not provided, but are not likely to substantially impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No data on deaths or health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926
Data Type:	DNA damage in vivo
HERO ID:	730538

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Low	× 1	3	No statistical analyses were performed (in vivo experiments). The figure legends indicate that data points represent means +/- standard deviations (SDs); however, SDs are inconsistently shown, and SDs for some groups are indistinguishable from other groups (thereby preventing estimation of these data for independent analyses).
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for most, but not all, outcomes were reported by exposure group. Findings were reported qualitatively in some cases (e.g., results from exposure of mice for 5 days).
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 27: **Animal toxicity evaluation results of Gocke et al 1981 for genetic mutations in Drosophila**

Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109					
Data Type:	mutations in Drosophila for DCM					
HERO ID:	20721					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively as dichloromethane.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch /lot number were not given; however, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Results for a cumulative negative control group were reported (Table 2). Multiple solvents were used for different test substances, and it was reported that "different solvents were used in separate controls."
	Metric 5:	Positive Controls	High	× 1	1	A concurrent positive control was used and a positive response was observed.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	The metric was not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared in 2% DMSO. No details on storage were provided; however, only a single application was used.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported as mM.
	Metric 10:	Exposure Frequency and Duration	Not Rated	NA	NA	More detailed methods were cited to other references.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two concentrations with approximately a 5-fold difference.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Species and strain were indicated. The source of the test strains 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:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type:	mutations in Drosophila for DCM
HERO ID:	20721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Not Rated	NA	NA	The adult feeding method was described in another publication.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The method reported and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Details of the outcome assessment protocol were cited in another paper (Wurgler et al., 1977).
	Metric 18:	Sampling Adequacy	High	× 1	1	About 1200 X-chromosomes were tested per experiment in each of 3 successive broods (3-3-4 days).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicale to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly (Kastenbaum-Bowman tables).
	Metric 24:	Reporting of Data	High	× 2	2	SLRL/chromosome tested and % were reported for each group.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 28: **Animal toxicity evaluation results of Hegi et al 1993 for p53 mutations in lung and liver tumors in mice**

Study Citation:	M. E. Hegi, P. Söderkvist, J. F. Foley, R. Schoonhoven, J. A. Swenberg, F. Kari, R. Maronpot, M. W. Anderson, R. W. Wiseman (1993). Characterization of p53 mutations in methylene chloride-induced lung tumors from B6C3F1 mice Carcinogenesis, 14(5,5), 803-810
Data Type:	p53 mutations in lung and liver tumors from DCM treated mice
HERO ID:	730544

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	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 3:	Test Substance Purity	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Analysis of lung tumor DNA from mice treated with DCM was compared with spontaneous lung tumors.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 9:	Reporting of Doses/Concentrations	Not Rated	NA	NA	Concentration was reported (2000 ppm), but more detailed methods such as analytical versus target concentrations were not included in the current reference. The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 hr/day, 5 days/wk for 2 years
	Metric 11:	Number of Exposure Groups and Dose Spacing	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.

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Study Citation:	M. E. Hegi, P. Söderkvist, J. F. Foley, R. Schoonhoven, J. A. Swenberg, F. Kari, R. Maronpot, M. W. Anderson, R. W. Wiseman (1993). Characterization of p53 mutations in methylene chloride-induced lung tumors from B6C3F1 mice Carcinogenesis, 14(5,5), 803-810
Data Type:	p53 mutations in lung and liver tumors from DCM treated mice
HERO ID:	730544

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 15:	Number per Group	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Several complimentary analyses were used including, loss of heterozygocity (LOH), single strand conformation polymorphism (SSCP), direct sequence analysis, Southern blotting, and immunohistochemistry.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assess 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.
	Metric 20:	Negative Control Response	High	× 1	1	Data were provide for spontaneous liver and lung tumors (no p53 mutations).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	The carcinogenicity bioassay that was the source of lung tumor DNA was cited in another publication.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 24:	Reporting of Data	Low	× 2	6	Representative data were 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 29: **Animal toxicity evaluation results of Lefevre and Ashby 1989 for inhalation study on DNA synthesis in mice**

Study Citation:	P. A. Lefevre, J. Ashby (1989). Evaluation of dichloromethane as an inducer of DNA synthesis in the B6C3F1 mouse liver Carcinogenesis, 10(6,6), 1067-1072
Data Type:	Inhalation - DNA synthesis in mouse liver
HERO ID:	730556

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified; batch and lot number were not identified; however, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	'Aristar' grade, minimum purity 99.8%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent controls were used (air for inhalation).
	Metric 5:	Positive Controls	Medium	× 1	2	Concurrent positive controls were used; however, phenobarbital was given by i.p. injection (not inhalation). Positive responses were observed.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly distributed.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Methods for the inhalation experiment were cited to another publication.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Methods for the inhalation experiment were cited to another publication.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Target inhalation concentration was reported without ambiguity. Information such as analytical/nominal concentrations and range of actual concentrations within a treatment group was not included.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single 2h exposure is adequate for the study design.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single concentration was employed (4000 ppm).
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Methods for the inhalation experiment were cited to another publication.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, lifestage, starting age and bw and commercial source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions such as food, water, lighting, and bedding were reported. Temperature and humidity were not given.
	Metric 15:	Number per Group	High	× 1	1	4-9/group is adequate.

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Study Citation:	P. A. Lefevre, J. Ashby (1989). Evaluation of dichloromethane as an inducer of DNA synthesis in the B6C3F1 mouse liver Carcinogenesis, 10(6,6), 1067-1072
Data Type:	Inhalation - DNA synthesis in mouse liver
HERO ID:	730556

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methods were well reported and are 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 concern.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of concern.
	Metric 20:	Negative Control Response	Low	× 1	3	One of the control groups in the inhalation experiment produced an "unusual response" (higher % S phases in hepatocytes than other controls).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported and DCM may produce an irritant reponse at 4000 ppm.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate (analysis of covariance following a logit transformation).
	Metric 24:	Reporting of Data	High	× 2	2	Individual animal data and cumulative data were reported.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 30: **Animal toxicity evaluation results of Lefevre and Ashby 1989 for gavage study on DNA synthesis in mice**

Study Citation:	P. A. Lefevre, J. Ashby (1989). Evaluation of dichloromethane as an inducer of DNA synthesis in the B6C3F1 mouse liver Carcinogenesis, 10(6,6), 1067-1072
Data Type:	Gavage - DNA synthesis in mouse liver
HERO ID:	730556

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified; batch and lot number were not identified; however, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	'Aristar' grade, minimum purity 99.8%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent controls were used (corn oil vehicle).
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls were used (TCE in corn oil via gavage). Positive responses were observed.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly distributed.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in corn oil was described. Storage was not indicated; however, a single gavage dose was used.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was consistent across groups and not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single gavage exposure is adequate for the study design.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose was employed (1000 mg/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 (gavage in corn oil).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Sprecies, strain, sex, lifestage, starting age and bw and commercial source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions such as food, water, lighting, and bedding were reported. Temperature and humidity were not given.
	Metric 15:	Number per Group	High	× 1	1	5/group is adequate.
Domain 5: Outcome Assessment						

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Study Citation:	P. A. Lefevre, J. Ashby (1989). Evaluation of dichloromethane as an inducer of DNA synthesis in the B6C3F1 mouse liver Carcinogenesis, 10(6,6), 1067-1072
Data Type:	Gavage - DNA synthesis in mouse liver
HERO ID:	730556

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methods were well reported and are 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 concern.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of concern.
	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	Food and water intake were not reported, but this is not considered to have had a significant impact on results given the short duration of the study (up to 48 hr).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate (analysis of covariance following a logit transformation).
	Metric 24:	Reporting of Data	High	× 2	2	Individual animal data and cumulative data were reported.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 31: **Animal toxicity evaluation results of Trueman and Ashby 1987 for unscheduled DNA synthesis in mouse and rat liver**

Study Citation:	R. W. Trueman, J. Ashby (1987). Lack of UDS activity in the livers of mice and rats exposed to dichloromethane Environmental and Molecular Mutagenesis, 10(2,2), 189-195
Data Type:	UDS in mouse and rat liver
HERO ID:	730588

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 was identified; no batch or lot number was given, but the composition is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be Aristar grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used (corn oil for gavage; control air for inhalation)
	Metric 5:	Positive Controls	High	× 1	1	Positive controls were used and responded appropriately (6BT for gavage; DEN in vitro for the air exposed controls for inhalation).
	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 and storage were reported and appropriate for gavage (dissolved in corn oil, administered immediately). 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	Exposures were administered consistently across study groups. Gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses and concentrations were reported without ambiguity. Target and analytical inhalation concentrations were reported and did not deviate videly (<10%). Analytical method was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure durations were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Two treatment groups plus control. Doses and concentrations were based on the cancer bioassay.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole body inhalation chamber; vapor may condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animals was not reported.

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Study Citation:	R. W. Trueman, J. Ashby (1987). Lack of UDS activity in the livers of mice and rats exposed to dichloromethane Environmental and Molecular Mutagenesis, 10(2,2), 189-195
Data Type:	UDS in mouse and rat liver
HERO ID:	730588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Low	× 1	3	The reported number of animals per study group was not sufficient for statistical analysis (varying numbers per group with some control groups consisting of only one animal; 3 analyzable animals per treatment group is recommended).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment method was partially reported and cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of minor details of outcome assessment protocol execution (e.g., grain counts).
	Metric 18:	Sampling Adequacy	Medium	× 1	2	25 to 50 cells examined, up to 3 slides/animal (100 cells recommended).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not relevant to the outcome of interest.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This metric is not relevant to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported and DCM is likely 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	Not Rated	NA	NA	Statistical analyses may not be required (OECD TG 486)
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups.
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 32: **Animal toxicity evaluation results of Sheldon et al 1987 for bone marrow micronucleus assay in mice**

Study Citation:	T. Sheldon, C. R. Richardson, B. M. Elliott (1987). Inactivity of methylene chloride in the mouse bone marrow micronucleus assay Mutagenesis, 2(1,1), 57-59
Data Type:	bone marrow MN in mice
HERO ID:	730594

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.
	Metric 2:	Test Substance Source	High	× 1	1	The source of test substance was reported as a manufacturer.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of the test substance was reported >99.8% and was adequate.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were reported and were solvent controls.
	Metric 5:	Positive Controls	High	× 1	1	Positive controls were reported and appropriate.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not described.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage of the test substance was reported and appropriate for the study.
	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 clearly and were appropriate for the study type.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration and frequency were reported and adequate for the study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of groups and spacing was based on a range finding study and was adequate for the study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route had limitations that were adequately addressed (dosing solutions were analyzed before and after dosing du to volatility).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics were reported and animals are routinely used for the study type. Starting body weight and health status were 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	High	× 1	1	Number of animals per group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						

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Study Citation:	T. Sheldon, C. R. Richardson, B. M. Elliott (1987). Inactivity of methylene chloride in the mouse bone marrow micronucleus assay Mutagenesis, 2(1,1), 57-59
Data Type:	bone marrow MN in mice
HERO ID:	730594

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologywas appropriate and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was inferred to be done consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (1000 PCEs examined).
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding of assessors was reported.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was appropriate for the study type.
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 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	Statistical methods were reported but not clearly described; however, data reported was sufficient for an independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and 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 33: **Animal toxicity evaluation results of Casanova et al 1996 for DNA binding in vivo**

Study Citation:	M. Casanova, R. B. Conolly, H. Heck (1996). DNA–protein cross-links (DPX) and cell proliferation in B6C3F1 mice but not Syrian golden hamsters exposed to dichloromethane: Pharmacokinetics and risk assessment with DPX as dosimeter Fundamental and Applied Toxicology, 31(1,1), 103-116
Data Type:	DNA binding in vivo for DCM
HERO ID:	730610

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as dichloromethane (DCM) and [14C]dichloromethane ([14C]DCM).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial sources of the test substances were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The radiochemical purity of the [14C]DCM was reported to be 99%. The purity of the unlabeled DCM was reported to be 99.9%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls are not needed for analysis of DNA binding (i.e., incorporation of radiolabeled DCM into DNA).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	No random allocation of animals was reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The preparation, handling, and storage of the test substance was described in detail and appropriate considering the volatility of the test substance.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent among treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in terms of ppm of DCM in the air. Concentrations of DCM were verified used gas chromatography of air samples taken from exposure chambers.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration was appropriate for the outcome of interest (6 hr/day for 2-3 days).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate for the test substances.
Domain 4: Test Organism						

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Study Citation:	M. Casanova, R. B. Conolly, H. Heck (1996). DNA–protein cross-links (DPX) and cell proliferation in B6C3F1 mice but not Syrian golden hamsters exposed to dichloromethane: Pharmacokinetics and risk assessment with DPX as dosimeter Fundamental and Applied Toxicology, 31(1,1), 103-116
Data Type:	DNA binding in vivo for DCM
HERO ID:	730610

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	The species, strain, sex, commercial source, age, and starting body weight range of both the mice and the hamsters were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Limited details about husbandry conditions were reported, but they appeared to be appropriate. The hamsters were exposed within 24 hours of their arrival, which is inappropriate; an acclimation period should have been included in the study design. This may have had a substantial impact on the study results.
	Metric 15:	Number per Group	Medium	× 1	2	The experiments were conducted with n = 2-3 mice per group and n = 3-4 hamsters per group. The group size of 2 mice per group for Table 4 is considered inadequate, but the remainder of the experiments were conducted with n = 3 mice.
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 was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	The number of technical replicates appears to vary throughout the experiments. Figure 3 specifies that each solid bar is the average of 3-4 groups of mice, each of which contained 3-4 mice. However, within the same graph, there was only one group of 3-4 hamsters, indicating 1 technical replicate per hamster.
	Metric 19:	Blinding of Assessors	High	× 1	1	This metric is not applicable to the study design.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls were not used.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported and DCM is likely to be an 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	No statistical analysis was performed on the data. However, summary data (mean ± SD) are provided in Tables 2 and 4, enabling independent statistical analysis.

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Study Citation:	M. Casanova, R. B. Conolly, H. Heck (1996). DNA–protein cross-links (DPX) and cell proliferation in B6C3F1 mice but not Syrian golden hamsters exposed to dichloromethane: Pharmacokinetics and risk assessment with DPX as dosimeter Fundamental and Applied Toxicology, 31(1,1), 103-116
Data Type:	DNA binding in vivo for DCM
HERO ID:	730610

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	The data were reported adequately.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 34: **Animal toxicity evaluation results of Rodriguez-Arnaiz 1998 for somatic mutation and recombination assay in Drosophila**

Study Citation:	R. Rodriguez-Arnaiz (1998). Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic w/w+ assay of Drosophila melanogaster Environmental and Molecular Mutagenesis, 31(4,4), 390-401
Data Type:	Somatic mutation and recombination assay in Drosophila
HERO ID:	732100

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, molecular and structural formula, and CASRN
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer. Batch/lot number and analytical verification were not reported, but the test substance is not expected to vary in composition.
	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 treated with solvent alone (3:1 ratio of ethanol:Tween 80) concurrently with experimental groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable to the study design (Drosophila).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation was reported, but storage was not. Test substance was dissolved in ethanol:tween 80 (3:1) and incorporated into feed upon which eggs were laid over the course of 3 days. No information was presented on the stability of the test material in the feed. It is unclear whether MeCl could have volatilized from the feed and/or been degraded.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	There was no indication that exposures were administered inconsistently; details of exposure administration were reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Target concentrations were reported as mM, presumably (but not specified) in feed.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Authors reported that eggs were laid on treated feed over 3 days (duration of larval stages) and newly hatched females were moved to fresh medium and scored 1-5 days later.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Four concentrations plus control were tested; dose spacing (50, 100, 250, and 500 mM) appears adequate.

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Study Citation:	R. Rodriguez-Arnaiz (1998). Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic w/w+ assay of Drosophila melanogaster Environmental and Molecular Mutagenesis, 31(4,4), 390-401
Data Type:	Somatic mutation and recombination assay in Drosophila
HERO ID:	732100

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	High	× 1	1	Feed is standard route for Drosophila
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	3 different strains of Drosophila were used. Commercial sources/crosses of the strains were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Little information on housing and culture conditions was provided. Temperature and humidity were provided.
	Metric 15:	Number per Group	Medium	× 1	2	Number per group not reported, but ~500 eyes were examined in most dose/strain groups
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Eyes examined for mosaic light spots under dissecting microscope.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Methods for assessing outcome (i.e, eye examination and classification of mosaic spots) were explained in detail.
	Metric 18:	Sampling Adequacy	High	× 1	1	~500 eyes per group were examined.
	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	Control response was reported and met expectations
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	This metric is not applicable to the study design (Drosophila).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis employed chi square for proportions.
	Metric 24:	Reporting of Data	Medium	× 2	4	Detailed results were reported , but standard deviations were not reported.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.4~~	
Extracted			Yes			

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Study Citation:	R. Rodriguez-Arnaiz (1998). Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic w/w+ assay of Drosophila melanogaster Environmental and Molecular Mutagenesis, 31(4,4), 390-401
Data Type:	Somatic mutation and recombination assay in Drosophila
HERO ID:	732100

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: "Suggest downgrading to medium because of uncertainty in the stability of the test material in the feed."

Table 35: **Animal toxicity evaluation results of Gocke et al 1981 for mouse micronucleus assay**

Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109					
Data Type:	mouse micronucleus assay for DCM and HCHO					
HERO ID:	20721					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified definitively by chemical name (dichloromethane and formaldehyde).
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch /lot number were not given; however, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported. Vehicle was olive oil for DCM and Hank's balanced salt solution (HBSS) for HCHO, but it is not known whether 0 mg/kg dose was a vehicle or untreated control group.
	Metric 5:	Positive Controls	Unacceptable	× 1	4	Positive controls were not used. Negative findings were observed for most compounds.
	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 prepared in olive oil for HBSS. No details on storage where provide however; the exposure duration was short.
	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 as mg/kg and mmol/kg.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration was appropriate for the outcome of interest. It was unclear why DCM was administered twice and HCHO once.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of dose groups was adequate (3 doses, plus control). Doses were not justified, but spacing seems adequate. Not clear if highest dose was high enough.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were suited to the test substance.
Domain 4: Test Organism						

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Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type:	mouse micronucleus assay for DCM and HCHO
HERO ID:	20721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animals were obtained from a commercial source and the test species/strain/sex was an appropriate animal model for the evaluation of the outcome(s) 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	2/sex/group was slightly lower than typical.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The method reported and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Details of the outcome assessment protocol were cited in another paper (Schmid, 1976).
	Metric 18:	Sampling Adequacy	Medium	× 1	2	The sampling was lacking at 1,000 erythrocytes from bone marrow per animal. Current standards are 4,000 erythrocytes per animal.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicale to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded as expected.
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, food/water intake, and/or respiratory rate is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly (Kastenbaum-Bowman tables).
	Metric 24:	Reporting of Data	Medium	× 2	4	Mean % micronucleated PE was reported for each group; variance was not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

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Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type:	mouse micronucleus assay for DCM and HCHO
HERO ID:	20721

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 36: **Animal toxicity evaluation results of Watanabe et al 2007 for intraperitoneal injection study in rats and mice on DNA adducts**

Study Citation:	K. Watanabe, R. G. Liberman, P. L. Skipper, S. R. Tannenbaum, F. P. Guengerich (2007). Analysis of DNA adducts formed in vivo in rats and mice from 1,2-dibromoethane, 1,2-dichloroethane, dibromomethane, and dichloromethane using HPLC/accelerator mass spectrometry and relevance to risk estimates Chemical Research in Toxicology, 20(11,11), 1594-1600
Data Type:	DNA adducts in rats and mice exposed i.p.
HERO ID:	732103

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified as 14C-dichloromethane ([14C]CH2CL2).
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance was obtained from a manufacturer, although it was unclear which of the two manufacturers listed was the source of the [14C]CH2CL2 specifically.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Study reports test material radiochemical purity was >95% with contamination primarily due to chloroform, determined by manufacturer using HPLC.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were utilized, but it was not clearly specified whether they were untreated or solvent-treated (PBS injection). It could be inferred that the negative controls were untreated due to lack of reporting PBS-only injections in the methods.
	Metric 5:	Positive Controls	High	× 1	1	It was specified that BrCH2CH2Br served as a positive control. BrCH2CH2Br yielded positive results.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report allocation methods.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported but storage was not; however, this is appropriate given the study design (single-dose i.p. administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Test substance administered as single dose by i.p. injection; no issues with test material administration were noted.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported both as mg/kg and uCi/kg
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Study reports single exposure, which is not unusual for adduct study

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Study Citation: K. Watanabe, R. G. Liberman, P. L. Skipper, S. R. Tannenbaum, F. P. Guengerich (2007). Analysis of DNA adducts formed in vivo in rats and mice from 1,2-dibromoethane, 1,2-dichloroethane, dibromomethane, and dichloromethane using HPLC/accelerator mass spectrometry and relevance to risk estimates Chemical Research in Toxicology, 20(11,11), 1594-1600
Data Type: DNA adducts in rats and mice exposed i.p.
HERO ID: 732103

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single dose was utilized, which is not unusual for an adduct study. However, it is unclear whether the dose utilized was high enough. Authors reported that limited availability of the test material "required that a limited amount of radioactivity be used per animal."
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were appropriate for the study design.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Male F344 rats and male and female B6C3F1 mice obtained from a commercial source were used; age and/or initial body weight were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Animal husbandry conditions were not reported; however, the animals were sacrificed 1 or 8 hours after dosing; thus, conditions were unlikely to impact the results .
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group is lacking at 2 animals per species per timepoint (1 and 8 hr). The data were not pooled for [14C}CH2CL2.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was described in detail, and efforts made to minimize processing time (due to instabiity of adducts) and loss of radioactivity (sealed vessels to prevent volatilization). Nucleoside adducts were separated using HPLC and major adduct standards; accelerator mass spec used to measure radioactivity.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Sacrifice and adduct measurements were made at 1 and 8 hrs after dosing.
	Metric 18:	Sampling Adequacy	High	× 1	1	Four DNA-GSH adducts were measured in liver and kidney (100 mg samples) from each animal.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not relevant for DNA adduct measurement
	Metric 20:	Negative Control Response	High	× 1	1	Background mean and SD radioactivity for untreated animals was reported in the text.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Confounding variables unlikely to impact results.

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Study Citation:	K. Watanabe, R. G. Liberman, P. L. Skipper, S. R. Tannenbaum, F. P. Guengerich (2007). Analysis of DNA adducts formed in vivo in rats and mice from 1,2-dibromoethane, 1,2-dichloroethane, dibromomethane, and dichloromethane using HPLC/accelerator mass spectrometry and relevance to risk estimates Chemical Research in Toxicology, 20(11,11), 1594-1600
Data Type:	DNA adducts in rats and mice exposed i.p.
HERO ID:	732103

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 were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical analysis was not performed due to most samples having no detectable radioactivity.
	Metric 24:	Reporting of Data	Low	× 2	6	Results reported qualitatively for MeCl.
Overall Quality Determination‡			~~High~~ ⟶ 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: "It is not clear that the doses of [14C]CH2Cl2 are high enough, and authors noted that the dose selection was dictated by the limited availability of the test substance. Negative results were obtained."

Table 37: **Animal toxicity evaluation results of Westbrook-Collins et al 1990 for intraperitoneal injection study in mice on sister chromatid exchanges**

Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81
Data Type:	SCEs and CAs in mice exposed i.p. - SCEs
HERO ID:	732105

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from manufacturer and was reported to be spectrophotometric grade; batch number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported as >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated vehicle and untreated control groups were included.
	Metric 5:	Positive Controls	High	× 1	1	Cyclophosphamide was tested as a positive control.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Study authors reported dissolving MeCl in corn oil immediately before use (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure details were provided and appeared to be consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported as mg/kg bw. injection volume was also reported and was not excessive.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure; acceptable for the endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Four dose groups plus control were tested (100, 1000, 1500, and 2000 mg/kg). High dose induced mortality in 2/4 animals and thus was likely too high.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	MeCl administered by i.p. injection; this route is less preferred based on available guidance for the study type
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were 3-5 month old male C57B1/6J mice obtained from Jackson Labs
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions including air changes, relative humidity, temperature, and light cycle were reported and appropriate, although RH slightly high (range 60-80%).
	Metric 15:	Number per Group	Medium	× 1	2	Four mice per dose were used; standard is 5 or more.

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Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81
Data Type:	SCEs and CAs in mice exposed i.p. - SCEs
HERO ID:	732105

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Methods were described as "standard cytogenetic methodology" and cited to other references.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Deaths occurred at 2000 mg/kg limiting the number of animals analyzed for SCEs at the high dose. However, there were 3 lower dose groups with no deaths.
	Metric 18:	Sampling Adequacy	High	× 1	1	30 second-division metaphases per animal evaluated for SCEs .
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were coded prior to analysis.
	Metric 20:	Negative Control Response	High	× 1	1	Control response was reported and as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No information regarding body weight or clinical signs was reported, although deaths at higher doses were observed. If increased SCEs were observed in this study, reporting clinical signs of toxicity would give more context to the doses at which SCEs were observed. However, due to the negative response, the lack of information regarding body weight or clinical signs is not expected to have impacted the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were not reported but sufficient data were reported to enable independent analysis for SCEs.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported in detail for SCEs (including mean, SD, and n).
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81
Data Type:	SCEs and CAs in mice exposed i.p. - SCEs
HERO ID:	732105

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 38: **Animal toxicity evaluation results of Westbrook-Collins et al 1990 for intraperitoneal injection study in mice on chromosome aberrations**

Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81					
Data Type:	SCEs and CAs in mice exposed i.p. - CAs					
HERO ID:	732105					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from manufacturer and was reported to be spectrophotometric grade; batch number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported as >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated vehicle and untreated control groups were included.
	Metric 5:	Positive Controls	High	× 1	1	Cyclophosphamide was tested as a positive control.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Study authors reported dissolving MeCl in corn oil immediately before use (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure details were provided and appeared to be consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported as mg/kg bw. injection volume was also reported and was not excessive.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure; acceptable for the endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Five dose groups plus control were tested (100, 500, 1000, 1500, and 2000 mg/kg). 1500 mg/kg and 2000 mg/kg induced mortality in 2/4 and 3/4 animals, respectively, and thus these doses were likely too high.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	MeCl administered by i.p. injection; this route is less preferred based on available guidance for the study type
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were 3-5 month old male C57B1/6J mice obtained from Jackson Labs
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions including air changes, relative humidity, temperature, and light cycle were reported and appropriate, although RH slightly high (range 60-80%).

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Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81
Data Type:	SCEs and CAs in mice exposed i.p. - CAs
HERO ID:	732105

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Four mice per dose were used; standard is 5 or more.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Methods were described as "standard cytogenetic methodology" and cited to other references.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Deaths occurred at 1500 and 2000 mg/kg limiting the number of animals analyzed for CAs at the high dose. However, there were 3 lower dose groups with no deaths.
	Metric 18:	Sampling Adequacy	Low	× 1	3	100 first division metaphases per animal assessed for CAs. Current standards recommend 200 metaphases per animal evaluated for CAs.
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were coded prior to analysis.
	Metric 20:	Negative Control Response	High	× 1	1	Control response was reported and as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No information regarding body weight or clinical signs was reported, although deaths at higher doses were observed. If increased CAs were observed in this study, reporting clinical signs of toxicity would give more context to the doses at which CAs were observed. However, due to the negative response, the lack of information regarding body weight or clinical signs is not expected to have impacted the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical analyses were not reported. Standard deviations were not reported for CAs, precluding statistical analysis. However, considering that the average number of CAs per cell in all of the DCM-exposed groups is lower than that of the uninjected control, and there is no dose response evident; therefore, statistical analysis is not critical to reaching a conclusion based on the results presented.
	Metric 24:	Reporting of Data	Medium	× 2	4	CA results presented as means without SDs.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	B. Westbrook-Collins, J. W. Allen, Y. Sharief, J. Campbell (1990). Further evidence that dichloromethane does not induce chromosome damage Journal of Applied Toxicology, 10(2,2), 79-81
Data Type:	SCEs and CAs in mice exposed i.p. - CAs
HERO ID:	732105

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 39: **Animal toxicity evaluation results of Suzuki et al 2014 for pig-a and gpt mutations, micronucleus and comet assay in mice**

Study Citation:	T. Suzuki, Y. Yanagiba, M. Suda, R. S. Wang (2014). Assessment of the genotoxicity of 1,2-dichloropropane and dichloromethane after individual and co-exposure by inhalation in mice Journal of Occupational Health, 56(3,3), 205-214
Data Type:	DCM pig-a and gpt mutations, MN and comet assay in mice
HERO ID:	2797857

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was reported by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was reported as a manufacturer.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported as 99.5%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control was exposed to air only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric was 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	Not Rated	NA	NA	The method and equipment used to generate the test substance as a vapor was cited to another publication.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported clearly and monitored by GC within 5% of the target concentrations.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposures groups and spacing was based on a previous toxicity study and appeared appropriate to evaluate the study outcomes.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported and obtained from a commercial source and are routinely used for the outcome.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Temperature and humidity were not reported.
	Metric 15:	Number per Group	High	× 1	1	Number of animals /group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						

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Study Citation:	T. Suzuki, Y. Yanagiba, M. Suda, R. S. Wang (2014). Assessment of the genotoxicity of 1,2-dichloropropane and dichloromethane after individual and co-exposure by inhalation in mice Journal of Occupational Health, 56(3,3), 205-214
Data Type:	DCM pig-a and gpt mutations, MN and comet assay in mice
HERO ID:	2797857

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methods were cited to several other publications.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methods were cited to several other publications.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to some outcomes; methods were cited to other publications.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type.
	Metric 20:	Negative Control Response		× 1	NA	The negative control response was appropriate for the outcome.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiration rate was not reported and may be a confounding variable in test design.
	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 for the data
	Metric 24:	Reporting of Data	High	× 2	2	data were presented for all outcomes and groups
Overall Quality Determination‡			High		0.0	
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 40: **Animal toxicity evaluation results of Hirata et al 2016 for 4- week oral study on in vivo mutagenicity, hepatic toxicity, and body weight**

Study Citation:	T. Hirata, Y. M. Cho, T. Toyoda, J. I. Akagi, I. Suzuki, A. Nishikawa, K. Ogawa (2016). Lack of in vivo mutagenicity of 1,2-dichloropropane and dichloromethane in the livers of gpt delta rats administered singly or in combination Journal of Applied Toxicology, 37(6,6), 683-691
Data Type:	Animal toxicity evaluation results of Hirata et al 2016 for 4- week oral study on in vivo mutagenicity, hepatic toxicity, and body weight
HERO ID:	3494227

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly stated
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source reported
	Metric 3:	Test Substance Purity	High	× 1	1	99.5% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control included
	Metric 5:	Positive Controls	Medium	× 1	2	As specified by OECD TG 488 a positive control is necessary The authors do mention a positive control response but only briefly and don't identify the dose used. Note that the need for positive control relates to the mutagenicity - which is related more to the mechanistic part of this study vs. the apical endpoints.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Radomization was stated, method based on body weights
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Dose groups prepared fresh prior to each dosing
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Administration consistent between groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses used were clearly stated, and based off of previous NTP study
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Daily for 4 weeks, which followed OECD TG 488
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Study included two doses and a control. The OECD TG 488 clearly states that 3 dose groups should be used if the limit dose of 1000 mg/kg-bw/day is not used. an MTD was not attained in this study - the doses could have included higher doses for a 28-day study.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure via gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The study used animals that were genetically modified (Gpt delta) as appropriate to the OECD TG 488. Note this rating is given for the mechanistic info (genetic toxicity) in this study.

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Study Citation:	T. Hirata, Y. M. Cho, T. Toyoda, J. I. Akagi, I. Suzuki, A. Nishikawa, K. Ogawa (2016). Lack of in vivo mutagenicity of 1,2-dichloropropane and dichloromethane in the livers of gpt delta rats administered singly or in combination Journal of Applied Toxicology, 37(6,6), 683-691
Data Type:	Animal toxicity evaluation results of Hirata et al 2016 for 4- week oral study on in vivo mutagenicity, hepatic toxicity, and body weight
HERO ID:	3494227

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry acceptable
	Metric 15:	Number per Group	High	× 1	1	7 animals/group; greater than specified by OECD TG 488
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Methods appropriate for the outcomes assessed
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies between groups
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals assessed for relevant outcomes
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only initial histology review and other non-subjective outcomes
	Metric 20:	Negative Control Response	Medium	× 1	2	several non-neoplastic liver lesions observed in control mice, but study authors indicate that many of the effects are common for this rat strain
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were not reported (although animals were randomly grouped based on body weight)
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No mortalities or sicknesses observed
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical analysis
	Metric 24:	Reporting of Data	High	× 2	2	Data reporting was adequate.
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 41: **Animal toxicity evaluation results of Andersen et al 2017 for gene expression in mouse lung and liver**

Study Citation:	M. E. Andersen, M. B. Black, J. L. Campbell, S. N. Pendse, H. J. Clewell, L. H. Pottenger, J. S. Bus, D. E. Dodd, D. C. Kemp, P. D. Mcmullen (2017). Combining transcriptomics and PBPK modeling indicates a primary role of hypoxia and altered circadian signaling in dichloromethane carcinogenicity in mouse lung and liver Toxicology and Applied Pharmacology, 332 149-158
Data Type:	Gene expression for DCM
HERO ID:	4032622

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was provided (a manufacturer). Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99.5%). The test substance purity was such that any observed effects were highly likely to be due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate negative control group (air-only) was used with all conditions equal except exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported that animals were randomly allocated into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The method and equipment used to generate the test substance as a vapor were reported and appropriate.
	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	Target and analytical chamber concentrations were reported, and actual concentrations were within 10% of target concentrations. The analytical method used to measure chamber test substance concentrations (gas chromatography) was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure (6 hours/day, 5 days/week for 13 weeks) were reported and appropriate for this study type. The study indicates that gene expression changes were evaluated in cancer target tissues after 90 days exposure for a number of chemical substances (to evaluate mode of action).

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Study Citation:	M. E. Andersen, M. B. Black, J. L. Campbell, S. N. Pendse, H. J. Clewell, L. H. Pottenger, J. S. Bus, D. E. Dodd, D. C. Kemp, P. D. Mcmullen (2017). Combining transcriptomics and PBPK modeling indicates a primary role of hypoxia and altered circadian signaling in dichloromethane carcinogenicity in mouse lung and liver Toxicology and Applied Pharmacology, 332 149-158
Data Type:	Gene expression for DCM
HERO ID:	4032622

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The study utilized 5 exposure groups plus controls. Concentrations appeared to have been chosen based on previous studies of DCM (including studies that showed evidence of carcinogenicity at 2000 ppm and above).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	A dynamic whole-body chamber was used for vapors that may condense. Airflow was maintained at 12-15 air changes per hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, age, and starting body weight were reported, and the test animal was obtained from a commercial source (Charles River Laboratories).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate for the study type.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported (n = 10 for treated groups; n = 5 for air controls), appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were addressed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	The study indicates that each series of arrays (for liver and lung tissues) was analyzed independently and included 4 biological replicates for each treatment condition (including controls). It is noted that some of the data for the in-life portion of the study uses n = 5 for controls (rather than n = 10).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This metric is not applicable to the study type (gene expression portion of the study). The control condition identifies baseline changes that serve as a basis of comparison for exposed groups. It is noted that biological responses of the negative control group was adequate for the in-life portion of the study.
Domain 6: Confounding / Variable Control						

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Study Citation:	M. E. Andersen, M. B. Black, J. L. Campbell, S. N. Pendse, H. J. Clewell, L. H. Pottenger, J. S. Bus, D. E. Dodd, D. C. Kemp, P. D. Mcmullen (2017). Combining transcriptomics and PBPK modeling indicates a primary role of hypoxia and altered circadian signaling in dichloromethane carcinogenicity in mouse lung and liver Toxicology and Applied Pharmacology, 332 149-158
Data Type:	Gene expression for DCM
HERO ID:	4032622

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	The inhalation study did not provide information on respiratory rate (DCM is expected to be a respiratory irritant).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Details regarding animal attrition and health outcomes unrelated to exposure were indirectly reported- the study indicated that none of the animals used in the study died; body weights were also similar among exposure groups. It is unlikely that health outcomes unrelated to exposure substantially impacted the study results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for datasets. Gene expression array files are available. It is noted that statistics was one of two criteria used to evaluate differential gene expression; for some analyses, a magnitude of change threshold was also applied (1.5-fold up- or down-regulation).
	Metric 24:	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 42: **In vitro evaluation results of Osterman-Golkar et al 1983 for bacterial reverse mutation**

Study Citation:	S. Osterman-Golkar, S. Hussain, S. Walles, B. Anderstam, K. Sigvardsson (1983). Chemical reactivity and mutagenicity of some dihalomethanes Chemico-Biological Interactions, 46(1,1), 121-130
Data Type:	Bacterial reverse mutation for DCM and formaldehyde
HERO ID:	9116

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as dichloromethane and formaldehyde.
	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 substances was not reported. Formaldehyde was considered an analytical reagent.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were included concurrently in study design. It is unclear whether these were treated with vehicle or left untreated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Concurrent positive control test substances were not included in the study design. Positive controls are routinely used for the Ames test, but are not required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay methods and procedures were briefly described. More detailed methods 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	Low	× 1	3	Preparation of the test substance was not reported. This is considered to have impacted results substantially, as DCM and formaldehyde are volatile compounds.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Administration of the test substances was reported to be consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Doses were not reported adequately. The authors note that "Differences in solubility and volatility makes it difficult to estimate the doses of the compounds[. . .]." This is considered to have seriously impacted the results of this study.
	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 adequate.

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Study Citation:	S. Osterman-Golkar, S. Hussain, S. Walles, B. Anderstam, K. Sigvardsson (1983). Chemical reactivity and mutagenicity of some dihalomethanes Chemico-Biological Interactions, 46(1,1), 121-130
Data Type:	Bacterial reverse mutation for DCM and formaldehyde
HERO ID:	9116

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	No metabolic activation was included, although this is routinely included in a bacterial reverse mutation assay. Formaldehyde was included as a metabolite of DCM.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. However, only the results from S. typhimurium TA100 were reported. Comparing the results from multiple strains is routine for the bacterial reverse mutation assay.
	Metric 15:	Number per Group	Low	× 1	3	The number of plates per treatment group was not reported (single may be acceptable).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of organisms used per group was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	The authors note "significant increases in mutation frequencies" at one point, but the statistical test utilized was not reported. It is also not clear whether there was >1 replicate per experimental condition, as only points (means) with no error bars are included in Figure 2a. Furthermore, raw data were not reported, although it may be possible to estimate from the graph.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity appears to have been assessed by observing reductions in the background lawn.

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Study Citation:	S. Osterman-Golkar, S. Hussain, S. Walles, B. Anderstam, K. Sigvardsson (1983). Chemical reactivity and mutagenicity of some dihalomethanes Chemico-Biological Interactions, 46(1,1), 121-130
Data Type:	Bacterial reverse mutation for DCM and formaldehyde
HERO ID:	9116

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Low	× 2	6	Data from only one bacterial strain are reported, although 3 strains are identified in the methods. Furthermore, a measurement of variation (e.g. standard deviation) is not included, assuming that >1 replicate was used.
Overall Quality Determination‡		Unacceptable⋆⋆		2.0	
Extracted		No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 43: **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 DCM
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 methylene chloride.
	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. Because toxicity was observed at the highest tested dose, data were available for only two analyzable concentrations.

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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						
	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 conversions, and mitotic recombinants.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analyses are not required by study type (data for individual plates were pooled, so that independent statistical analyses are not possible). Data were presented as the number of revertants,recombinants, or convertants per 10^5 survivors (pooled data); data for numbers of revertants, recombinants, or convertants per plate (and including a measure of variation) were not reported.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive result was explicitly specified (i.e., at least a doubling of colonies compared to the controls).
	Metric 24:	Cytotoxicity Data	High	× 1	1	A measure of cytotoxicity (percent survival compared to control, measured by total number of colonies counted) was determined concurrently with the mutagenicity assay results.

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

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 44: **In vitro evaluation results of Thier et al 1993 for reverse mutation in bacteria transfected with GSH transferase**

Study Citation:	R. Thier, J. B. Taylor, S. E. Pemble, W. G. Humphreys, M. Persmark, B. Ketterer, F. P. Guengerich (1993). Expression of mammalian glutathione S-transferase 5-5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to dihalomethanes Proceedings of the National Academy of Sciences, 90(18,18), 8576-8580
Data Type:	Reverse mutation in bacteria transfected with GSH transferase for DCM
HERO ID:	12093

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as CH2CL2 (dichloromethane; DCM).
	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	Low	× 2	6	Negative controls were included. It is unclear whether these were solvent treated or left untreated, and it is unclear whether they were concurrent with the treated plates. However, more detailed methods were cited to other publications.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not included; however, this is a mechanistic study using multiple compounds and positive dose response relationships were observed.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described. More detailed methods were cited to other publications, but appeared appropriate.
	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	Test substance preparation was reported adequately. 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	Exposure was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were not stated, but can be determined from the x axis of Figure 3d.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The authors note a pre-incubation time of 5 minutes, which is shorter than current guidelines (48-72 hr), but it is unclear whether there was a subsequent exposure (e.g. direct plate incorporation exposure). More detailed methods were cited to other publications.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.

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Study Citation:	R. Thier, J. B. Taylor, S. E. Pemble, W. G. Humphreys, M. Persmark, B. Ketterer, F. P. Guengerich (1993). Expression of mammalian glutathione S-transferase 5-5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to dihalomethanes Proceedings of the National Academy of Sciences, 90(18,18), 8576-8580
Data Type:	Reverse mutation in bacteria transfected with GSH transferase for DCM
HERO ID:	12093

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. Rather than using liver S9, this study utilized transfection of bacterial with a functional GSH transferase enzyme to activate the test substance.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The S. typhimurium strain TA1535 is commonly used for the outcome of interest. The transfected strain was characterized and found to have similar spontaneous mutation rates to the original strain and retained other key characteristics.
	Metric 15:	Number per Group	Not Rated	NA	NA	It was unclear how many replicates per experimental conditions were utilized. More detailed methods were cited to other publications.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate and sensitive 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	Low	× 2	6	The initial number of organisms was not reported for each 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	Low	× 1	3	It does not appear that the data were analyzed statistically. It is unclear what the criteria for a positive result were, but the authors do reference dose-dependence. The data could be estimated from Figure 3d, but variance is not reported (assuming that >1 replicate per experimental condition was used).
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines.

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Study Citation:	R. Thier, J. B. Taylor, S. E. Pemble, W. G. Humphreys, M. Persmark, B. Ketterer, F. P. Guengerich (1993). Expression of mammalian glutathione S-transferase 5-5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to dihalomethanes Proceedings of the National Academy of Sciences, 90(18,18), 8576-8580
Data Type:	Reverse mutation in bacteria transfected with GSH transferase for DCM
HERO ID:	12093

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Low	× 1	3	It does not appear that cytotoxicity was accounted for in the study design; however, it does not appear that a reduction in the background lawn was observed at higher doses. Assessing cytotoxicity is common but not required for the bacterial reverse mutation assay.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported adequately, although it is possible that a measurement of variance (e.g. standard deviation) was not given if replicates were >1 per experimental condition.
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 45: **In vitro evaluation results of Gocke et al 1981 for bacterial reverse mutation**

Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type:	Bacterial reverse mutation
HERO ID:	20721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances was identified definitively as dichloromethane and formaldehyde.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch /lot number were not given; however, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A negative control group was inferred by reference to "spontaneous frequency of revertants"; however, no details were provided.
	Metric 5:	Positive Controls	Medium	× 2	4	The use of positive controls was indicated in the text. It appears that benzo[a]pyrene was utilized as a positive control. It is unclear what concentration(s) of B[a]P was/were tested, but it yielded positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited in other publications, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Information on preparation and storage was not reported, but methods for this assay were cited to other publications.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Details on application methods were cited to other publications.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations for DCM were reported as ug/plate. Concentrations for HCHO were not specified.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Methodology details were cited to other publications.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Reported as 5 doses, usually up to 3600 ug/plate (no further details).

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Study Citation: E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type: Bacterial reverse mutation
HERO ID: 20721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system (e.g., S9 from aroclor-, -induced rats) was reported in the study; however, details regarding composition mix, concentration, or quality control information were not described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test strains were reported with limited descriptive information.
	Metric 15:	Number per Group	Not Rated	NA	NA	Method details were cited to other publications.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method addressed and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported.
	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.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of organisms per replicate per group was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not described clearly (Kastenbaum-Bowman tables).
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance and dose dependency ("concentration response") were alluded to as criteria for a positive result, but no further details were provided.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Low	× 2	6	Data were presented graphically for only 2 strains. Data for HCHO were not presented graphically. It was indicated in the summary table that HCHO yielded a negative response.

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Study Citation:	E. Gocke, M. T. King, K. Eckhardt, D. Wild (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities] Mutation Research, 90(2,2), 91-109
Data Type:	Bacterial reverse mutation
HERO ID:	20721

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Unacceptable⋆⋆		2.2	
Extracted		No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 46: **In vitro evaluation results of Green 1983 for bacterial reverse mutation**

Study Citation:	T. Green (1983). The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium Mutation Research: Genetic Toxicology, 118(4,4), 227-288
Data Type:	Bacterial reverse mutation DCM
HERO ID:	29110

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Dichloromethane was identified by chemical name, deuterated DCM was also tested.
	Metric 2:	Test Substance Source	High	× 1	1	DCM was obtained from BDH Chemicals Limited and deuterated DCM was obtained from Fluke A.G. Cofactors. Batch/lot number not reported but not required for these chemicals.
	Metric 3:	Test Substance Purity	High	× 1	1	AnalaR grade for DCM; 99% pure for deuterated DCM
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative air controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Chlorofluoromethane was also tested using these same methods, and produced a positive response; however, it is not clear whether this compound would be considered a positive control for volatile halogenated compounds.
	Metric 6:	Assay Procedures	Medium	× 1	2	A modified Ames et al. (1975) procedure was used and the modifications were briefly described. Details were lacking (pre-incubation temperatures, cell density culture media, humidity, washing/rinsing methods, 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	Medium	× 1	2	Preparation of the test substance for vapor exposure was briefly described (known volume of volatile dihalomethanes were injected into jar for evaporation to create a measured concentration in the atmosphere of the jar). Storage information was not provided.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported, and atmospheres were analyzed by gas chromatography during incubation. However, it was not reported if exposures were consistent across study groups and based on this method of exposure, inconsistencies could have occurred in exposure groups.

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Study Citation:	T. Green (1983). The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium Mutation Research: Genetic Toxicology, 118(4,4), 227-288
Data Type:	Bacterial reverse mutation DCM
HERO ID:	29110

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	2.8% and 8.4% v/v concentrations were tested, however, the middle concentration was only reported on a graph (approximately 5%; Figure 1). It is unclear if these were averages of the concentrations measured by gas chromatography. 2.8% v/v concentration was tested for deuterated DCM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Reported up to 3 days (72 hours). Additional measurements appear to have been collected at approximately 3, 6, 12, and 24 hours at the 2.8% v/v concentration for DCM (Figure 3).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Justification for using 3 concentrations and the concentrations chosen were not reported (only 1 concentration for deuterated DCM).
	Metric 13:	Metabolic Activation	Medium	× 1	2	Conducted in the presence and absence of metabolic activation. Several metabolic activation systems were used (S9, Cytosol, Microsomes, and boiled S9). Preparation was briefly described. Quality control methods were not described. Metabolic activation volume was 0.2 mL. It is unclear if cytosol was used undiluted in this experiment. Numerical results were only reported for 2.8% v/v, other concentration results were in a graph (Figures 1 and 3). Information on the preparation of boiled S9 was not provided.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Only a single strain of Salmonella typhimurium was used (TA100). It was not reported if the test model was a commercial source or laboratory-maintained.
	Metric 15:	Number per Group	High	× 1	1	5 plates/concentration were used and is appropriate for a reverse mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Assessed number of revertants per plate in bacteria mutagenicity assay.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Results for all concentrations were provided in a graph (Figure 1) and a brief summary, however, additional, more detailed results were provided for the 2.8% v/v concentration that were not provided for the other 2 concentrations tested (DCM). It is unclear if results exist for the other two concentrations tested.
	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.

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Study Citation:	T. Green (1983). The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium Mutation Research: Genetic Toxicology, 118(4,4), 227-288
Data Type:	Bacterial reverse mutation DCM
HERO ID:	29110

Domain	Metric		Rating†	MWF⋆	Score	Comments††
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 analysis was not reported and statistically significant results (per concentration) were not identified, however, Table 1 provides enough information to calculate significant results independently (2.8% v/v concentration only).
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria was not reported.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not assessed, but may not be required for the outcome.
	Metric 25:	Reporting of Data	Low	× 2	6	Numerical results were reported for the 2.8% v/v concentration, and results for the other two concentrations were provided in a graph (Figure 1). General mutagenic results were summarized, however, were not provided for each concentration both with and without metabolic activation.
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 47: **In vitro evaluation results of Jongen et al 1978 for bacterial reverse mutation**

Study Citation:	W. M. F. Jongen, G. M. Alink, J. H. Koeman (1978). Mutagenic effect of dichloromethane on Salmonella typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 56(3,3), 245-248
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29117

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as dichloromethane.
	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	Low	× 2	6	Negative controls were included in the study design. It is unclear if the negative controls were concurrent and whether they were vehicle-treated or left untreated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. The use of positive controls in the bacterial reverse mutation assay is common but not required.
	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 the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation, handling, and storage of the volatile test substance was reported and appropriate.
	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 in terms of ppm. Concentrations on DCM in culture media was not determined. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (48 hr at 37°C).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was appropriate (5). The dose spacing was somewhat lacking as it covered just one order of magnitude. Cytotoxicity was apparent at the highest dose.

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Study Citation:	W. M. F. Jongen, G. M. Alink, J. H. Koeman (1978). Mutagenic effect of dichloromethane on Salmonella typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 56(3,3), 245-248
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29117

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was used, although the amount of liver S9 added was not specified. More detailed methods were cited to other publications.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Identity and origin of two S. typhimurium strains, TA98 and TA100, were reported. These strains are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was carried out in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this endpoint.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initials conditions were not reported for each group or replicate.
	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	No statistical analysis was conducted; however, mean and standard deviation were provided for each experimental condition.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines. A positive result was not specifically defined, but was related to increased revertants and dose-dependency.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	A measurement of cytotoxicity was conducted (although not concurrently) and methods were briefly described.
	Metric 25:	Reporting of Data	Medium	× 2	4	Average revertants per plate for each experimental condition were reported with a measurement of variation (unclear whether this represented standard deviation or standard error of the mean).

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Study Citation:	W. M. F. Jongen, G. M. Alink, J. H. Koeman (1978). Mutagenic effect of dichloromethane on Salmonella typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 56(3,3), 245-248
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29117

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 48: **In vitro evaluation results of Jongen et al 1982 for bacterial reverse mutation**

Study Citation:	W. M. F. Jongen, E. G. M. Harmsen, G. M. Alink, J. H. Koeman (1982). The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 95(2-3,2-3), 183-189
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29118

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 dichloromethane (DCM).
	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 purity of the test substance was not reported, but it was noted that the test substance was of analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls (air-exposed) were included in the study design both with and without metabolic activation.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. The use of positive controls in the bacterial reverse mutation assay is common but not required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay methods and procedures were very briefly described. More detailed methods 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	Details regarding preparation, storage, and exposure of the volatile test substance were cited to other references.
	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 in terms of % DCM in atmosphere. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (6 hr). Guidelines for the outcome of interest include exposure durations of 48-72 hr. However, a positive response was observed, indicating that the 6 hr duration was sufficient.

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Study Citation:	W. M. F. Jongen, E. G. M. Harmsen, G. M. Alink, J. H. Koeman (1982). The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 95(2-3,2-3), 183-189
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29118

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups was adequate for the study type; however, there was no indication of cytotoxicity at the highest tested concentration, which is contrary to current guidelines for the bacterial reverse mutation assay. It is unclear how the concentrations were selected.
	Metric 13:	Metabolic Activation	High	× 1	1	Phenobarbital-induced rat liver S9 was utilized. The source, method of preparation, and concentration of the rat liver S9 fraction was reported. Aroclor-induced rat liver S9 was also used, but at only one concentration of DCM in a supplementary experiment with the goal of comparing the two metabolic activation methods.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	S. typhimurium strain TA100 was used in the study design, which is routinely used for the outcome of interest. Multiple strains are commonly used for the bacterial reverse mutation assay. The source of the bacterial cultures was not reported.
	Metric 15:	Number per Group	High	× 1	1	Triplicate plating was used for the experiment of interest (Figure 1A).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this endpoint.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of organisms per group or replicate was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						

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Study Citation:	W. M. F. Jongen, E. G. M. Harmsen, G. M. Alink, J. H. Koeman (1982). The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 95(2-3,2-3), 183-189
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	29118

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistics were not used to assess increased revertants/plate, either from control or comparing with/without metabolic activation. A positive result was not specifically defined, but it was suggested that it was related to increased revertants and dose-dependency. Only means (with no measure of variance, e.g. standard deviation) were included in Figure 1A, so independent statistical analysis could not be performed. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) were reported and appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	No measurement or indication of cytotoxicity was included. Given that positive results were observed (i.e. the exclusion of cytotoxicity measurement did not result in a false negative), this is considered acceptable.
	Metric 25:	Reporting of Data	Medium	× 2	4	Standard deviations were not provided.
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 49: **In vitro evaluation results of Jongen et al 1981 for unscheduled DNA synthesis assay**

Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Unscheduled DNA synthesis assay for DCM
HERO ID:	29119

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 dichloromethane (DCM).
	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 purity of the test substance was not reported, but it was noted that the test substance was of analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included in the study design. The identity of the negative controls (i.e. solvent or untreated) is not specified for this endpoint, but it is inferred based on other endpoints in this article that it is a solvent control.
	Metric 5:	Positive Controls	High	× 2	2	The positive control included in the study design, 4-nitro-quinoline-N-oxide (4NQO), was appropriate for the outcome of interest.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	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	Details regarding preparation, storage, and exposure of the volatile test substance were described adequately.
	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 in terms of % DCM in atmosphere. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.
	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 groups and dose spacing were adequate for the outcome of interest.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Unscheduled DNA synthesis assay for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	Low	× 2	6	Both Chinese hamster epithelial cells (V79) and primary human fibroblasts (AH) were used for this endpoint. The culture medium and propagation methods were reported, but no other details were provided (such as origin of the cells or doubling times). More detailed information is required for primary human fibroblasts specifically, such as demographic information of the donor (age, gender), health status of the donor, method of isolation (biopsy or postmortem collection), organ of origin (e.g. lung, skin), and method of propagation. Use of primary human cells also necessitates use of multiple strains, i.e. from multiple donors, to account for intra-species variability (typically not required for immortalized cell lines). The lack of details regarding the primary human fibroblasts utilized here is considered to be a substantial limitation. However, it should be noted that V79 cells are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Unacceptable	× 2	8	Current guidelines for the in vivo UDS assay specify that at least 100 non-S-phase nuclei be scored per replicate. Previous guidelines for the in vitro UDS assay specify that at least 50 non-S-phase nuclei be scored per replicate. The present experiment assessed 25 nuclei of non-S-phase cells per dose level. This is considered to be inadequate and unacceptable.
	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	Initial conditions were not reported for each group or replicate.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Unscheduled DNA synthesis assay for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No statistical analysis was conducted on the data and raw data are not provided. Independent statistical analysis may be conducted by estimating means and standard error of the mean from the graphs in Figure 4.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported and appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A cytotoxicity test was conducted, although not concurrently with the sister chromatid exchange assay. The cytotoxicity test involved an incubation period of 7 days after a 1-hour exposure to DCM in the same manner as in the SCE assay. Cytotoxicity was shown at the higher concentrations of DCM.
	Metric 25:	Reporting of Data	High	× 2	2	Raw data were not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			Yes			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 50: **In vitro evaluation results of Jongen et al 1981 for DNA synthesis**

Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	DNA synthesis for DCM
HERO ID:	29119

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 dichloromethane (DCM).
	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 purity of the test substance was not reported, but it was noted that the test substance was of analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included in the study design; however, identity of the negative controls (i.e. solvent or untreated) was not specified.
	Metric 5:	Positive Controls	High	× 2	2	The positive control included in the study design, 4-nitro-quinoline-N-oxide (4NQO), was appropriate for the outcome of interest.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and/or cited in another publication(s), but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Details regarding preparation, storage, and exposure of the volatile test substance were described adequately.
	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 in terms of % DCM in atmosphere. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.
	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 groups and dose spacing were adequate for the outcomes of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	DNA synthesis for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Both Chinese hamster epithelial cells (V79) and primary human fibroblasts (AH) were used for this endpoint. The culture medium and propagation methods were reported, but no other details were provided (such as origin of the cells or doubling times). More detailed information is required for primary human fibroblasts specifically, such as demographic information of the donor (age, gender), health status of the donor, method of isolation (biopsy or post-mortem collection), organ of origin (e.g. lung, skin), and method of propagation. Use of primary human cells also necessitates use of multiple strains, i.e. from multiple donors, to account for intra-species variability (typically not required for immortalized cell lines). The lack of details regarding the primary human fibroblasts utilized here is considered to be a substantial limitation.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies are 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	Low	× 2	6	The initial number of cells per group or replicate were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	No statistical analysis was conducted on the data; however, statistics may not have been necessary (mean of only 2 replicates). Variance was provided for UDS, but not DNA synthesis data.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	DNA synthesis for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported and appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	A cytotoxicity test was conducted, although not concurrently with the UDS or DNA synthesis assays. The cytotoxicity test involved an incubation period of 7 days after a 1-hour exposure to DCM. Cytotoxicity was assessed in V79 and CHO cells, but not the primary human fibroblasts also used in the UDS and DNA synthesis assays. Therefore, it is unknown whether DCM was cytotoxic to the primary human fibroblasts and, if so, at what concentrations. Cytotoxicity was shown at the higher concentrations of DCM in V79 and CHO cells.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		1.6	
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 51: **In vitro evaluation results of Jongen et al 1981 for sister chromatid exchange**

Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Sister chromatid exchange for DCM
HERO ID:	29119

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 dichloromethane (DCM).
	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 purity of the test substance was not reported, but it was noted that the test substance was of analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls (2% DMSO) were included in the study design. This concentration of DMSO is somewhat high, but the study included trials of the sister chromatid exchange assay with 1%, 2%, and 3% DMSO that indicated no increase in exchange rate, so this was considered to be an acceptable negative control.
	Metric 5:	Positive Controls	High	× 2	2	The positive control included in the study design, 4-nitro-quinoline-N-oxide (4NQO), was appropriate for the outcome of interest.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	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	Two methods of exposure to the test substance were used in this study. It appears that both methods of exposure were used for the sister chromatid exchange assay, but it is unclear which was used for the data shown in Figure 1 and Table 1. Details regarding preparation of the volatile test substance were adequate.
	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 in terms of % DCM in atmosphere. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Sister chromatid exchange for DCM
HERO ID:	29119

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 (1 hr).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and dose spacing were adequate for the outcome of interest.
	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	Medium	× 2	4	Chinese hamster epithelial cells (V79) were used for this endpoint. The culture medium and propagation methods were reported, but no other details were provided (such as origin of the cells or doubling time). This model is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 25 metaphases/replicate.
	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 differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Both Student's t-test and ANOVA were utilized to analyze the sister chromatid exchange data. Student's t-test was initially used for the first experiment to compare results of each concentration to control. In follow-up experiments, ANOVA (with no post-hoc test) was utilized to detect variation within each experiment as well as among experiments. Because mean and SEM are provided, independent statistical analysis could be conducted on these data.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Sister chromatid exchange for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of SCEs/chromosome) were reported and appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A cytotoxicity test was conducted, although not concurrently with the sister chromatid exchange assay. The cytotoxicity test involved an incubation period of 7 days after a 1-hour exposure to DCM in the same manner as in the SCE assay. Cytotoxicity was shown at the higher concentrations of DCM.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
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 Jongen et al 1981 for mammalian HGPRT forward mutation assay**

Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	29119

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 dichloromethane (DCM).
	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 purity of the test substance was not reported, but it was noted that the test substance was of analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included in the study design. The identity of the negative controls (i.e. solvent or untreated) is not specified for this endpoint.
	Metric 5:	Positive Controls	Low	× 2	6	The positive control included in the study design, ethyl methanesulfonate (EMS), was appropriate for the outcome of interest. However, the positive control was only conducted with V79 cells, rather than both V79 and CHO cells. Furthermore, the exposure duration of 17 hours to EMS was substantially longer than the exposure duration to the DCM (1 hour). Therefore, the positive controls used in this assay are considered to have significant limitations.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and/or cited in another publication(s), but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Two methods of exposure to the test substance were used in this study. It appears that both methods of exposure were used for the HGPRT assay, but it is unclear which was used for the data shown in Figures 2 and 3. Details regarding preparation of the volatile test substance were adequate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in terms of % DCM in atmosphere. It is inferred that these are nominal concentrations, as no validation of the atmospheric concentrations was reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration was shorter than current guidelines recommend (1 hour versus 3-6 hours). This is considered to have had a substantial impact on results. Furthermore, the positive control groups were exposed to EMS for 17 hours, and no justification is provided for the substantial difference between DCM-treated and positive control exposure durations.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and dose spacing were adequate for the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	No metabolic activation condition was included. This is contrary to current guidelines; given that a negative result was obtained without metabolic activation, experimental conditions with metabolic activation should be included to verify the result.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Both Chinese hamster epithelial cells (V79) and Chinese hamster ovary cells (CHO) were used for this endpoint. The culture medium and propagation methods were reported, but no other details were provided (such as origin of the cells or doubling times). These models are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable 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	Initial conditions (e.g., number of cells) were not reported for each group or replicate.

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Study Citation:	W. M. F. Jongen, P. H. M. Lohman, M. J. Kottenhagen, G. M. Alink, F. Berends, J. H. Koeman (1981). Mutagenicity testing of dichloromethane in short-term mammalian test systems Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 81(2,2), 203-213
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	29119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	No statistical analysis was conducted on the data; however statistics may not be warranted for means that represent duplicates only.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported and appropriate for the outcome of interest.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A cytotoxicity test was conducted concurrently with the HGPRT mutation assay. Cytotoxicity was shown at the higher concentrations of DCM.
	Metric 25:	Reporting of Data		× 2	NA	Data for exposure-related findings were presented for all outcomes 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 53: **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 DCM
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 dichloromethane.
	Metric 2:	Test Substance Source	Medium	× 1	2	The sources of the test substances used in the study were identified (from Carlo Erba, Milan, Italy or Merck-Schuchardt), but it was unclear which test substances originated from which source.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of test substances used in the study ranged from 97-99% (purity of individual test substances not specified).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were described adequately (e.g., cell density, volumes,temperature). The in vitro system used was partially cited to another publication.
	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	The preparation of the test substance was reported; however, it was not explicitly indicated that microtest plates were covered (re: volatility of the test substance). Although the storage of the test substance was not reported, this omission is unlikely to 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	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.

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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 DCM
HERO ID: 75278

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	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 did not induce 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.
	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						

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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 DCM
HERO ID:	75278

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 54: **In vitro evaluation results of Thilagar and Kumaroo 1983 for sister chromatid exchange in Chinese hamster ovary cells**

Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	SCE in CHO cells, methylene chloride
HERO ID:	93655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from Fisher Sci. cert A.C.S lot 713580
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported, but it was noted that the test substance was certified ACS grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were included, but it was not specified whether these were solvent-treated or untreated.
	Metric 5:	Positive Controls	Medium	× 2	4	Positive control triethylenemelamine and cyclophosphamide were tested concurrently. It was not specified whether each were treated with or without metabolic activation. However, it can be inferred that cyclophosphamide was in the presence of metabolic activation, given that it is an indirect-acting compound. Both yielded positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods were described and appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation and storage of the test substance were not reported. Given the volatility of the test substance, this is considered to have potentially impacted results substantially.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Doses were administered consistently across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations for the SCE assay were reported in Table 1 without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration with activation was 2h exposure to test substance followed by growth in medium for 24h. Exposure duration without activation was continuous exposure to the test substance for 24h. These exposure durations are considered adequate for the SCE assay.

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Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	SCE in CHO cells, methylene chloride
HERO ID:	93655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of groups was 3-4 concentrations plus controls and was based on cytotoxicity found in a range finding study. Number of groups and spacing were appropriate for the study type
	Metric 13:	Metabolic Activation	High	× 1	1	Aroclor induced rat liver S9 was used as metabolic activation, described and previously cited, and is commonly used
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model is reported, was obtained from a commercial source and is routinely used for the outcome of interest
	Metric 15:	Number per Group	High	× 1	1	Assays were run in duplicate in both plastic and glass flasks and the number of cells were 4 x 10ˆ5/flask and was appropriate for the study type
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	The outcome assessment was carried out consistently across concentration groups
	Metric 18:	Sampling Adequacy	High	× 2	2	evaluated 25/culture or 50 total and appropriate for the outcome of interest
	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	There were no confounding variables that influenced the outcome assessment
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	With the reported interaction of the test substance with the plastic flasks, glass flasks were used in duplicate for each test group
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was reported and is appropriate for the study type
	Metric 23:	Data Interpretation	High	× 2	2	Scoring criteria was reported and is consistent with established criteria
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was determined in a range finding study and evaluated by viable cell count, cells/flask, and relative cell growth

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Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	SCE in CHO cells, methylene chloride
HERO ID:	93655

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported for all outcomes and exposure groups
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 55: **In vitro evaluation results of Thilagar and Kumaroo 1983 for chromosomal abnormalities in Chinese hamster ovary cells**

Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	CA in CHO cells, methylene chloride
HERO ID:	93655

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from Fisher Sci. cert A.C.S lot 713580
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported, but it was noted that the test substance was certified ACS grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were included, but it was not specified whether these were solvent-treated or untreated.
	Metric 5:	Positive Controls	Medium	× 2	4	Positive control triethylenemelamine and cyclophosphamide were tested concurrently. It was not specified whether each were treated with or without metabolic activation. However, it can be inferred that cyclophosphamide was in the presence of metabolic activation, given that it is an indirect-acting compound. Both yielded positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods were described and appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation and storage of the test substance were not reported. Given the volatility of the test substance, this is considered to have potentially impacted results substantially.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Doses were administered consistently across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations for the CA assay were reported in Table 2 without ambiguity.

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Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	CA in CHO cells, methylene chloride
HERO ID:	93655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration with activation was 2h exposure to test substance followed by growth in medium for 12h. Exposure duration without activation was continuous exposure to the test substance for 12 hr. This is acceptable, but the exposure time of 2h with metabolic activation is somewhat lacking (current standards 3-6 hours). Given the positive results in the CA assay, this is not expected to have substantially impacted results.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of groups was 3-4 concentrations plus controls and was based on cytotoxicity found in a range finding study. Number of groups and spacing were appropriate for the study type
	Metric 13:	Metabolic Activation	High	× 1	1	Aroclor induced rat liver S9 was used as metabolic activation, described and previously cited, and is commonly used
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model is reported, was obtained from a commercial source and is routinely used for the outcome of interest
	Metric 15:	Number per Group	High	× 1	1	Assays were run in duplicate in both plastic and glass flasks and the number of cells were 4 x 10^5/flask and was appropriate for the study type
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	The outcome assessment was carried out consistently across concentration groups
	Metric 18:	Sampling Adequacy	Medium	× 2	4	Evaluated 100/treatment; less than guidance (300 well-spread metaphases per concentration), but clearly positive and appropriate for the outcome of interest
	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	There were no confounding variables that influenced the outcome assessment

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Study Citation:	A. K. Thilagar, V. Kumaroo (1983). Induction of chromosome damage by methylene chloride in CHO cells DNA Repair, 116(3-4,3-4), 361-367
Data Type:	CA in CHO cells, methylene chloride
HERO ID:	93655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	With the reported interaction of the test substance with the plastic flasks, glass flasks were used in duplicate for each test group
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was reported and is appropriate for the study type
	Metric 23:	Data Interpretation	High	× 2	2	Scoring criteria was reported and is consistent with established criteria
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was determined in a range finding study and evaluated by viable cell count, cells/flask, and relative cell growth
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and exposure 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 56: **Animal toxicity evaluation results of Allen et al 1990 for inhalation and subcutaneous injection studies in mice for chromosome aberrations and sister chromatid exchanges in bone marrow**

Study Citation:	J. Allen, A. Kligerman, J. Campbell, B. Westbrook-Collins, G. Erexson, F. Kari, E. Zeiger (1990). Cytogenetic analyses of mice exposed to dichloromethane Environmental and Molecular Mutagenesis, 15(4,4), 221-228
Data Type:	inhalation and s.c. injection-CA, MN, SCE assays
HERO ID:	29217

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, synonym and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported and analytical confirmation was performed by elemental analysis and GC.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used for each experiment (air for inhalation, corn oil for s.c. injection).
	Metric 5:	Positive Controls	Medium	× 1	2	DMBA was used as a positive control for SCE and CA following s.c. injection and a response was observed. It is unclear whether a positive control is available via the inhalation route.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The method and equipment used to generate the test substance as a vapor were reported and appropriate. For the s.c. injection experiment, prepartion in corn oil was described (single injection).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups. Injection volume was consistent and not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations and doses were reported without ambiguity. Weekly mean vapor concentrations were within 10% of the target concentations at all positions sampled in the chambers. The analytical method was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Inhalation experiments were conducted for 6h/day, 5 days/wk for 2 or 12 wk. Single s.c. injection.

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Study Citation:	J. Allen, A. Kligerman, J. Campbell, B. Westbrook-Collins, G. Erexson, F. Kari, E. Zeiger (1990). Cytogenetic analyses of mice exposed to dichloromethane Environmental and Molecular Mutagenesis, 15(4,4), 221-228
Data Type:	inhalation and s.c. injection-CA, MN, SCE assays
HERO ID:	29217

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	2 exposure groups were were used for the 10-day inhalation and s.c. injection experiments. Only one exposed group was used for the 12-wk inhalation study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole-body chambers were used; DCM may condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, age and source were reported. Health status 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.
	Metric 15:	Number per Group	High	× 1	1	4-10/group is appropriate for the outcome of interest,
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods reported and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the cytogentic assays (CAs for 200 metaphases/animal; lung MN 1,000 cells/group; erythrocyte MN 2000 PCE; SCE 50 cells/animal).
	Metric 19:	Blinding of Assessors	High	× 1	1	It was noted that all slides were coded prior to analysis.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported. DCM is expected to be an irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group; however, this is not considered to have substantially impacted results.
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 for exposure-related findings were presented for all outcomes by exposure group.

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Study Citation:	J. Allen, A. Kligerman, J. Campbell, B. Westbrook-Collins, G. Erexson, F. Kari, E. Zeiger (1990). Cytogenetic analyses of mice exposed to dichloromethane Environmental and Molecular Mutagenesis, 15(4,4), 221-228
Data Type:	inhalation and s.c. injection-CA, MN, SCE assays
HERO ID:	29217

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 57: **In vitro evaluation results of Doherty et al 1996 for micronucleus assay**

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance is clearly identified by name (carbon tetrachloride).
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance was not obtained from a manufacturer, but was supplied as a gift (from Dr. R. Crebelli in Rome). Although there did not appear to be analytical verification of the test substance in this study, this study cited publications by Dr. Crebelli (including studies of chlorinated hydrocarbons).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The report indicates that the study authors used concurrent negative control groups (vehicle was indicated to be culture medium). It appears that all conditions were equal except exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the response for CCl4 (and other chemicals) was positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods and procedures (including cell density, culture media, incubation temperatures, washing/rinsing methods, and slide preparation) were decribed. Details of some procedures (e.g., kinetochore labeling) were cited to other publications. Although procedures deviated somewhat from customary practices, they appeared to be applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation conditions were reported. It was indicated that, owing to insolubility of the test substances (in general), stock solutions were prepared in growth medium at the top concentration to be tested and were placed in an incubator (with shaking) overnight, and then diluted. It was not specified what methods were conducted to minimize loss of the volatile test substance, but it was noted that the exposures were carried out in glass vials, which were assumed to be closed systems for the duration of the exposure; therefore, this is not considered to have substantially impacted the results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type. It was noted that, owing to the protocol being used (i.e., use of genetically modified cell lines rather than S9), the exposure duration could be extended to encompass the whole cell cycle (18 hours for AHH-1 cells and 24 hours for MCL-5 and h2E1 cell lines).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (4 plus control) and concentration spacing were considered adequate to address the purpose of the study (e.g., evaluation of exposure-response relationships). Concentrations up to 10 mM were used, which is standard for studies of this type.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study was conducted using metabolically competent cells (rather than an exogenous activation system). The parental cell line used in the study (AHH-1) had only a low level of native CYP1A1 activity; the other two cell lines enabled activation via additional CYP enzymes (CYP2E1 for h2E1 cells, and CYP2E1, 1A2, 2A6, 3A4 and epoxide hydrolase). The study states that genetically modified cells lines such as those used in this study have been shown in other studies to detect metabolites produced from indirect-acting compounds.
Domain 4: Test Model						

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

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

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	The study indicates that significant effects (with respect to micronuclei induction) reported in the results and discussion were based on significance in the Chi-squared test at the 99% confidence limit. The results section describes statistically significantly increased micronuclei formation in the various cell lines, largely without reference to specific exposure levels. The accompanying table (Table I-ix for CCl4) and figures do not provide indications of statistical significance; however, raw data are provided, enabling independent statistical analysis. The "lowest significant dose" of induction of kinetochore positive/negative nuclei (from replicate experiments) was provided in an additional table (Table II).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study authors alluded to (but did not explicitly report) the evaluation criteria (i.e., a statistically significantly increase in micronuclei); the evaluation criteria are consistent with studies of this type.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study indicates that relative toxicity was evaluated as the proportion of binucleate and mononucleate cells; the proportion of binucleate cells provides an estimate of the nuclear cell division index and this a measure of toxicity. Although the assessment of cytotoxicity was not fully described/accounted for, these omissions are not likely to substantially impact the study results. For example, toxicity at 10 mM CCl4 in all cell lines appeared to be >55% relative to the negative control; however, micronuclei formation was seen at lower exposure concentrations in the absence of substantial (relative) toxicity.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related outcomes were reported by exposure group.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 58: **In vitro evaluation results of Doherty et al 1996 for micronucleus assay**

Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay_DCM
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 (methylene chloride).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The test substance was obtained from a manufacturer. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The report indicates that the study authors used concurrent negative control groups (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 DCM (and other chemicals) was positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods and procedures (including cell density, culture media, incubation temperatures, washing/rinsing methods, and slide preparation) were decribed. Details of some procedures (e.g., kinetochore labeling) were cited to other publications. Although procedures deviated somewhat from customary practices, they appeared to be applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation conditions were reported. It was indicated that, owing to insolubility of the test substances (in general), stock solutions were prepared in growth medium at the top concentration to be tested and were placed in an incubator (with shaking) overnight, and then diluted. It was not specified what methods were conducted to minimize loss of the volatile test substance, but it was noted that the exposures were carried out in glass vials, which were assumed to be closed systems for the duration of the exposure; therefore, this is not considered to have substantially impacted the results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type. It was noted that, owing to the protocol being used (i.e., use of genetically modified cell lines rather than S9), the exposure duration could be extended to encompass the whole cell cycle (18 hours for AHH-1 cells and 24 hours for MCL-5 and h2E1 cell lines).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (4 plus control) and concentration spacing were considered adequate to address the purpose of the study (e.g., evaluation of exposure-response relationships). Concentrations up to 10 mM were used, which is standard for studies of this type.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study was conducted using metabolically competent cells (rather than an exogenous activation system). The parental cell line used in the study (AHH-1) had only a low level of native CYP1A1 activity; the other two cell lines enabled activation via additional CYP enzymes (CYP2E1 for h2E1 cells, and CYP2E1, 1A2, 2A6, 3A4 and epoxide hydrolase). The study states that genetically modified cells lines such as those used in this study have been shown in other studies to detect metabolites produced from indirect-acting compounds.
Domain 4: Test Model						

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

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

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	The study indicates that significant effects (with respect to micronuclei induction) reported in the results and discussion were based on significance in the Chi-squared test at the 99% confidence limit. The results section describes statistically significantly increased micronuclei formation in the various cell lines, largely without reference to specific exposure levels. The accompanying table (Table I-viii for DCM) 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, the toxicity at 10 mM DCM in AHH-1 cells appeared to be >55% relative to the negative control; however, no evidence of micronuclei formation was seen across the range of administered exposure cocentrations. Toxicity in the other two cells lines was < 55% (standard for this study type); micronuclei formation was seen 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		1.2	
Extracted			Yes			

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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_DCM
HERO ID:	194804

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 59: **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 - DCM
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 dichloromethane (75-09-2); CH2CL2.
	Metric 2:	Test Substance Source	Medium	× 1	2	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.

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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 - DCM
HERO ID:	194882

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 liver induced with Aroclor-1254). Volume in the final culture was provided. 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.
	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 and sensitive 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, but the method of measurement was not explicitly described.
	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		1.4	
Extracted			Yes			

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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 - DCM
HERO ID:	194882

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 60: **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 DCM
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 dichloromethane with the correct CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of DCM 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 DCM 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 DCM 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.

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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 DCM
HERO ID:	194949

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model					
Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest.
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 and senditive 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			

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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 DCM
HERO ID:	194949

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 61: **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_ DCM
HERO ID:	200282

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was clearly identified by name (dichloromethane). According to EPA's System of Registries, the CASRN provided (1665-00-5) corresponds to "dichloromethane-d2"/"dideuteromethylenechloride".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (purchased from Fluka AC Buchs). 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%); 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 DCM 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						

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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_ DCM
HERO ID:	200282

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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.
	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).

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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_ DCM
HERO ID:	200282

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	Minor 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 1826 in controls to 310 in the highest exposure group (presumably due to decreased germination at higher concentrations). The lowest number of colonies scored was at the lowest concentration (279 colonies scored in 0.08% DCM group). It is unclear why the colony count was low for this exposure group.
	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						
	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; 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.

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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_ DCM
HERO ID:	200282

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 62: **In vitro evaluation results of Oda et al 1996 for SOS/umu test in S. typhimurium**

Study Citation:	Y. Oda, H. Yamazaki, R. Thier, B. Ketterer, F. P. Guengerich, T. Shimada (1996). A new Salmonella typhimurium NM5004 strain expressing rat glutathione S-transferase 5-5: Use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system Carcinogenesis, 17(2,2), 297-302
Data Type:	SOS/umu test in S. typhimurium
HERO ID:	200516

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as methylene dichloride (CH2Cl2).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Wako Pure Chemical Industries). 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 explicitly reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group; it appears that all conditions were the same except exposure to the test substance. The vehicle for the test substances was DMSO, but it was unclear whether negative controls were untreated or DMSO-treated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods and procedures were briefly described and partially cited to another publication from the same laboratory (Oda et al. 1993). The methods used appeared to be appropriate to the study type. The expressed B-galactosidase activity was determined by a method cited to another reference.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The study indicated that bacterial suspensions were incubated with the test substance (20 uL dissolved in DMSO) for 2 hours. Given the short-term nature of the experiment, reporting test substance storage was not necessary.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	In so much as details were reported, it appeared that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported adequately. The doses were not explicitly stated, but could be determined by estimation from Figure 3D.

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Study Citation: Y. Oda, H. Yamazaki, R. Thier, B. Ketterer, F. P. Guengerich, T. Shimada (1996). A new Salmonella typhimurium NM5004 strain expressing rat glutathione S-transferase 5-5: Use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system Carcinogenesis, 17(2,2), 297-302
Data Type: SOS/umu test in S. typhimurium
HERO ID: 200516

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appeared to be appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	An adequate number of groups was used (4 + control) and the spacing of the dose groups was adequate for the evaluation of a dose-response. The higher doses were high enough to observe cytotoxicity, as determined by cell growth %.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The source of the parental strain (S.typhimurium TA 1535/pSK1002) was not reported. S.typhimurium TA1535/pSK1002 and a strain generated by the laboratory for this experiment (S. typhimurium NM5004) were utilized. The study authors performed experiments to validate the genotype of the new strain (i.e., evaluating GST 5-5 and umuC expression).
	Metric 15:	Number per Group	High	× 1	1	The study authors indicate that each point in Figure 3 is the mean for two or three independent experiments. Therefore, the number of replicates per study group (2 or 3) were appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (cellular beta-galactosidase activity as a measure of umuC gene expression) addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment protocol was applied consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						

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Study Citation: Y. Oda, H. Yamazaki, R. Thier, B. Ketterer, F. P. Guengerich, T. Shimada (1996). A new Salmonella typhimurium NM5004 strain expressing rat glutathione S-transferase 5-5: Use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system Carcinogenesis, 17(2,2), 297-302
Data Type: SOS/umu test in S. typhimurium
HERO ID: 200516

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were minor differences with respect to study group parameters; the study results are not likely to be substantially impacted. The spontaneous level of umuC gene expression in the parental S.typimurium TA1535/pSK1002 strain (123+/-15 units; without addition of test substance) appeared to be slightly elevated relative to the NM5004 strain (97+/-15 units)n. No statistics were performed; the difference was less than 2SD.
	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	Unacceptable	× 1	4	Statistical analyses were not performed, and data were not provided for independent analyses. The data presented in Figure 3 are the mean for 2-3 independent experiments; no measure of variation was provided (even graphically).
	Metric 23:	Data Interpretation	Medium	× 2	4	The evaluation criteria were partially reported. A positive response is increased umuC expression (without reference to a specific magnitude of change), and a negative result is no change in umuC expression. With respect to DCM, the study referenced a dose-related increase in umuC expression; therefore, the dose-relatedness of the effect is presumably one of the criteria for a positive effect (this was not explicitly specified).
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was measured as decreased cell growth; however, methods of measurements were not fully described/reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related outcomes were reported by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

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Study Citation:	Y. Oda, H. Yamazaki, R. Thier, B. Ketterer, F. P. Guengerich, T. Shimada (1996). A new Salmonella typhimurium NM5004 strain expressing rat glutathione S-transferase 5-5: Use in detection of genotoxicity of dihaloalkanes using an SOS/umu test system Carcinogenesis, 17(2,2), 297-302
Data Type:	SOS/umu test in S. typhimurium
HERO ID:	200516

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 63: **In vitro evaluation results of Simula et al 1993 for in vitro mutagenicity assay in S. typhimurium**

Study Citation:	T. P. Simula, M. J. Glancey, C. R. Wolf (1993). Human glutathione S-transferase-expressing Salmonella typhimurium tester strains to study the activation/detoxification of mutagenic compounds: Studies with halogenated compounds, aromatic amines and aflatoxin B1 Carcinogenesis, 14(7,7), 1371-1376
Data Type:	In vitro mutagenicity assay assay in S. typhimurium
HERO ID:	200592

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.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (BDH). 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 test substance was analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study reported using negative control groups, for which all conditions except exposure to the test substance appeared to be equal.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although traditional positive control substances were not used, the study evaluated the ability of GST enzymes to modulate mutagenicity of known mutagens. The response for methylene dichloride (and other chemicals used in the study) was positive and concentration-related.
	Metric 6:	Assay Procedures	Medium	× 1	2	In general, assay methods and procedures were described in adequate detail (partially cited to modified procedure presented in another publication).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was partially reported (vapor generation information not specified); plates were incubated with the vaporized test substance in (sealed) glass jars to account for the volatility of the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were shown (in Figure 3) without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (incubated for 16 hours in exposure chambers) and was considered appropriate for the study type.

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Study Citation: T. P. Simula, M. J. Glancey, C. R. Wolf (1993). Human glutathione S-transferase-expressing Salmonella typhimurium tester strains to study the activation/detoxification of mutagenic compounds: Studies with halogenated compounds, aromatic amines and aflatoxin B1 Carcinogenesis, 14(7,7), 1371-1376

Data Type: In vitro mutagenicity assay assay in S. typhimurium

HERO ID: 200592

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Three exposure concentration plus control were used (at least 5 analyzable concentrations are recommended). No rationale was provided for the concentrations tested; however, the exposure levels were adequate to show results relevant to the outcome of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. Tests were conducted in the absence of activation and in bacterial strains that expressed human GST enzymes.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and their sources were reported. Salmonella typhimurium strains were obtained from a commercial source and are routinely used for the outcome of interest. The generation of Salmonella strains expressing human GSTs was described in detail, and the expression of GSTs in these strains was verified by SDS-PAGE.
	Metric 15:	Number per Group	Unacceptable	× 1	4	Based on the data provided in Figure 3, it appears that only one replicate/bacterial strain was used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (i.e., numbers of revertant colonies) addressed the outcome of interest (i.e., mutagenicity).
	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	This metric not applicable to the study type.
	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	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	T. P. Simula, M. J. Glancey, C. R. Wolf (1993). Human glutathione S-transferase-expressing Salmonella typhimurium tester strains to study the activation/detoxification of mutagenic compounds: Studies with halogenated compounds, aromatic amines and aflatoxin B1 Carcinogenesis, 14(7,7), 1371-1376
Data Type:	In vitro mutagenicity assay assay in S. typhimurium
HERO ID:	200592

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analyses are not required for this study type (and not possible if 1 replicate/strain was used). Data are provided graphically, enabling an independent analysis of the study result (e.g., evaluation of a 2-fold increased number of revertants).
	Metric 23:	Data Interpretation	Medium	× 2	4	The evaluation criteria (i.e., quantification of revertants) was considered appropriate. It was inferred from the text that a dose-related increase in the numbers of revertants was considered a positive result.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	High	× 2	2	Outcomes were reported by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		1.4	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 64: **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 DCM
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 methylene chloride.
	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 (presumably including DCM) 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).

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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 DCM
HERO ID: 626038

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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.
	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 DCM.
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 DCM in Table 1 are shown as the percentage of control.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not reported.

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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 DCM
HERO ID:	626038

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined in the study report, and methods used for assessing cytotoxicity were described (i.e., trypan dye exclusion). However, it is not clear if there were cytotoxicity data for DCM (no data were shown in Table 1). It is inferred from the text that there was not substantial toxicity because the test substance would have been tested at lower doses if cytotoxicity had been observed.
	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⋆⋆		1.7	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 65: **In vitro evaluation results of Graves et al 1994 for bacterial reverse mutation in S. typhimurium**

Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial reverse mutation in S. typhimurium for DCM
HERO ID:	626445

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 and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The specific purity of the test substance was not reported, but it was HPLC 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	× 2	2	The positive control used for S. typhimurium (MNNG) was reported and appropriate. A positive response was observed from positive controls.
	Metric 6:	Assay Procedures	Low	× 1	3	Assay procedures for S. typhimurium strains were largely cited to other references (limited information provided in study).
	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	Preparation and handling of the volatile test substance was partially described and partially cited to other references. It was indicated that exposure occurred in air-tight glass jars; omissions related to handling of the volatile test substance are not likely to impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The amount of test substance used in the experiment was provided without ambiguity (in mL DCM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (72 hours).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Four doses plus a control were used (at least 5 analyzable concentrations are recommended).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.

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Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial reverse mutation in S. typhimurium for DCM
HERO ID:	626445

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The source of Salmonella typhimurium strain TA 100 was not reported; however, the strain is routinely used in bacterial mutagenicity assays. The source of S. typhimurium strain NG-11 (derived from TA 100) was specified (gift from another lab). Although little descriptive information was provided, these omissions are not likely to impact the study results.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted with n = 5 replicates for DCM.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study 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	High	× 1	1	No statistical analysis was conducted on the data. Summary data (mean with standard deviation) could be estimated from Figure 1, enabling independent statistical analysis.
	Metric 23:	Data Interpretation	Medium	× 2	4	It is inferred from the text that a 2-fold change was considered a positive response.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were not defined for the Salmonella portion of the study; the test substance was presumably toxic at the highest tested dose (as evidenced by lower numbers of revertants).
	Metric 25:	Reporting of Data	High	× 2	2	Outcomes were reported by exposure group.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial reverse mutation in S. typhimurium for DCM
HERO ID:	626445

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 66: **In vitro evaluation results of Graves et al 1994 for bacterial reverse mutation in E. coli**

Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial mutation in E. coli for DCM
HERO ID:	626445

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 and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The specific purity of the test substance was not reported, but it was HPLC 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	× 2	2	The positive controls used (mitomycin C and MMS without S9; NDMA with S9) were appropriate; positive responses were observed. It is noted that no volatile positive control substances were used.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures for E. coli strains were described adequately.
	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 and handling of the volatile test substance was described adequately. It was stated that the test substance was injected through a Teflon seal into flasks (i.e., sealed containers were used).
	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	Concentrations were reported without ambiguity. Owing to the volatility of the test substance, concentrations were confirmed by gas chromatography analysis. Aqueous concentrations of DCM for E. coli exposure corresponding to 2.5, 5, or 10 µL DCM were 30, 60, and 130 mM, respectively.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (2 hours). Given the positive response of the cells after this exposure, the duration is considered adequate.

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Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial mutation in E. coli for DCM
HERO ID:	626445

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study used three analyzable concentrations plus a control (at least 5 analyzable concentrations recommended).
	Metric 13:	Metabolic Activation	Medium	× 1	2	Mouse and rat liver S9 fractions were included as experimental conditions, but they were not induced (e.g. by Aroclor or phenobarbital). Methods of preparation was cited to other references.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Wild-type and DNA repair deficient strains of E. coli strains were utilized. The uvrA strain is routinely used for the bacterial mutagenicity assay. The source of these strains was reported (i.e., another laboratory).
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study 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	High	× 1	1	No statistical analysis was conducted on the data. Summary data (mean with standard deviations) were provided, enabling independent statistical analysis.
	Metric 23:	Data Interpretation	Medium	× 2	4	It is inferred from the text that a 2-fold change in revertant colonies was considered a positive response.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was assessed and methods for the cytotoxicity assessment were reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.

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Study Citation:	R. J. Graves, R. D. Callander, T. Green (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride Mutation Research, 320(3,3), 235-243
Data Type:	Bacterial mutation in E. coli for DCM
HERO ID:	626445

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 67: **In vitro evaluation results of Graves et al 1996 for mammalian HGPRT forward mutation assay**

Study Citation:	R. J. Graves, P. Trueman, S. Jones, T. Green (1996). DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: Comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde Mutagenesis, 11(3,3), 229-233
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	626446

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 methylene chloride.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance 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	High	× 1	1	The test substance was reported to be 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included in the study design. The identity of the negative controls (i.e. solvent or untreated) was not specified, but it was inferred based on this group serving as a negative control for all treatments (multiple chemicals) that it is an untreated control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was included in the study design (and not strictly required). The test substance induced a positive response in the presence of activation (type of control recommended by guideline).
	Metric 6:	Assay Procedures	Medium	× 1	2	Details of the experiment were outlined briefly, but included basic information such as cell density and test conditions. Detailed methods 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	Medium	× 1	2	Preparation of the test substance was reported in minimal detail; more detailed methods were cited to other references. Although the test substance is volatile, it appears that this omission did not substantially impact the study results (flasks were presumably sealed).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	R. J. Graves, P. Trueman, S. Jones, T. Green (1996). DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: Comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde Mutagenesis, 11(3,3), 229-233
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	626446

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The dose was reported in terms of % DCM in the media. It is noted that the methods cite a DCM concentration of 0.3%, and the results in Table 1 indicate that the concentration was 0.25%.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was shorter than current standards and guidelines recommend (1 hour versus 3 to 6 hours). However, considering the positive result from the DCM exposure, this is not considered to have substantially impacted results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Only one dose of DCM was included in the study design. However, the purpose of the study was to examine the types of mutations induced by DCM (rather than evaluate the dose-response); therefore, the dose used appears to be appropriate to address the outcome of interest.10-20%
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study authors report utilizing a commonly used metabolic activation system (mouse liver S100 fraction); however, few details were provided. Given that the fraction was obtained from a commercial source, this omission is not likely to substantially impact the study results.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity, commercial source, and culture methods of the Chinese hamster ovary cells were reported. This cell line is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each mutation experiment was repeated at least 3 times. Results from these data were pooled (rather than presented separately).
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.

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Study Citation:	R. J. Graves, P. Trueman, S. Jones, T. Green (1996). DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: Comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde Mutagenesis, 11(3,3), 229-233
Data Type:	Mammalian HGPRT forward mutation assay for DCM
HERO ID:	626446

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	High	× 1	1	No statistical analysis was conducted on the data. Summary data (mean and standard error of the mean) are provided and enable independent statistical analysis.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not explicitly reported, but it is inferred from the text that a several-fold increase in mutation frequency was considered a positive response.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Plating efficiency was determined concurrently with the mutation assay. Methods used for evaluating cytotoxicity were defined and described adequately.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 68: **In vitro evaluation results of Zeiger 1990 for bacterial reverse mutation with vapor phase in dessicator**

Study Citation:	E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type:	Bacterial reverse mutation (vapor phase in dessicator) for DCM
HERO ID:	629923

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance reported by name, structure and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source of the test substance was 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	Negative control was reported as an untreated control (no solvent; appropriate for the vapor exposure).
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were reported and were appropriate for the study type. 4-nitro-o-phenylenediamine is not a standard positive control without metabolic activation for TA98, but positive responses were observed.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described and were appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation was well described and accounted for the volatility of the test substance. 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 consistent across study groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were clearly reported in ml/chamber. The volume of the chamber was reported to be 9 liters. It is not known whether the entire volume of the liquid test substance volatilized during the 24 hour exposure duration, but given the length of the exposure, this is not considered to have impacted the results substantially.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration was reported, but less than standard (48-72 hours) however, it was considered appropriate for the vapor phase dessicator study design.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was appropriate and spacing appeared was not justified but appeared adequate to show outcome of interest

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Study Citation: E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type: Bacterial reverse mutation (vapor phase in dessicator) for DCM
HERO ID: 629923

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic activation was used aroclor 1254 derived from rat (SD) and Hamster (Syrian) liver. This is commonly used for they study type
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was briefly described and is routinely used for the outcome of interest
	Metric 15:	Number per Group	Medium	× 1	2	Number per group was not reported. Given the standard deviations reported, it is inferred that at least duplicate plates were utilized for each experimental condition, which is adequate for the bacterial reverse mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology was appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables 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	Low	× 1	3	No statistical analysis was conducted. Summary data were only partially provided (mean and standard deviation, but not sample size), so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial mutation assay.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were briefly described by category based on magnitude of increased revertants and shape of dose response and are consistent with guidelines
	Metric 24:	Cytotoxicity Data	Low	× 1	3	No cytotoxicity data were reported; however, it could be inferred from the drop in number of revertants at the highest dose in comparison to the second-highest dose that cytotoxicity was observed at the highest dose in the dessicator protocol (Table Vb).

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Study Citation:	E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type:	Bacterial reverse mutation (vapor phase in dessicator) for DCM
HERO ID:	629923

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported for all outcomes and exposure 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 69: **In vitro evaluation results of Zeiger 1990 for bacterial reverse mutation with standard preincubation**

Study Citation:	E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type:	Bacterial reverse mutation (standard preincubation) for DCM
HERO ID:	629923

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance reported by name, structure and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source of the test substance was 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	Negative control was reported as solvent (DMSO) control and was appropriate for the study type.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were reported and were appropriate for the study type. 4-nitro-o-phenylenediamine is not a standard positive control without metabolic activation for TA98, but positive responses were observed.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were previously cited (preincubation protocol) and were assumed to be appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Preparation, including accounting for test substance volatility, is assumed to be in the cited references.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	More detailed methods were cited to other references.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were clearly reported in ug/plate
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was appropriate and spacing appeared was not justified but appeared adequate to show outcome of interest
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic activation was used aroclor 1254 derived from rat (SD) and Hamster (Syrian) liver. This is commonly used for they study type
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was briefly described and is routinely used for the outcome of interest

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Study Citation:	E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type:	Bacterial reverse mutation (standard preincubation) for DCM
HERO ID:	629923

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Not Rated	NA	NA	Number per group was not reported but assumed to be previously cited and appropriate for the study type. Given the standard deviations reported, it is inferred that at least duplicate plates were utilized for each experimental condition, which is adequate for the bacterial reverse mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology was appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables 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	Low	× 1	3	No statistical analysis was conducted. Summary data were only partially provided (mean and standard deviation, but not sample size), so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial mutation assay.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were briefly described by category based on magnitude of increased revertants and shape of dose response and are consistent with guidelines
	Metric 24:	Cytotoxicity Data	Low	× 1	3	No cytotoxicity data were reported; however, it could be inferred from the drop in number of revertants at the highest dose in comparison to the second-highest dose that cytotoxicity was observed at the highest dose in the dessicator protocol (Table Vb).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and exposure groups
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	E. Zeiger (1990). Mutagenicity of 42 chemicals in Salmonella Environmental and Molecular Mutagenesis, 16(S18,S18), 32-54
Data Type:	Bacterial reverse mutation (standard preincubation) for DCM
HERO ID:	629923

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: **In vitro evaluation results of Demarini et al 1997 for mutagenicity assay**

Study Citation:	D. M. Demarini, M. L. Shelton, S. H. Warren, T. M. Ross, J. Y. Shim, A. M. Richard, R. A. Pegram (1997). Glutathione S-transferase-mediated induction of GC->AT transitions by halomethanes in Salmonella Environmental and Molecular Mutagenesis, 30(4,4), 440-447
Data Type:	Mutagenicity assay
HERO ID:	657294

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 (dichloromethane; CH2Cl2).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (EM Science). 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 (99.99%) was such that any observed effects were highly likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The use of a negative controls was reported. Presumably, all conditions were equal except for exposure to the test substance (i.e. sterile air), but this was not explicitly specified. These omissions are not expected to significantly impact the study results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control was not used (not absolutely required for studies of this type).
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Some methods and procedures were cited to other publications (i.e., Pegram et al. 1997; Hughes et al. 1987). However, methods used to account for the volatility of the test substance were described (albeit briefly).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Details with respect to the preparation of the test substance were reported in sufficient detail. The study indicated how vapors were generated, and indicated that DCM was prepared in sealed containers.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	The study suggests that exposures were administered consistently across groups.

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Study Citation:	D. M. Demarini, M. L. Shelton, S. H. Warren, T. M. Ross, J. Y. Shim, A. M. Richard, R. A. Pegram (1997). Glutathione S-transferase-mediated induction of GC->AT transitions by halomethanes in Salmonella Environmental and Molecular Mutagenesis, 30(4,4), 440-447
Data Type:	Mutagenicity assay
HERO ID:	657294

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The study reports that each chemical was evaluated at at least 3 doses; however, Table 1 only provides one of the doses per strain, which was described as "a dose that produced a response at the highest point of the linear portion of the dose-response curve," (i.e. highest dose tested that did not produce cytotoxicity. This is considered to be acceptable given that this single dose was reported without ambiguity. Other doses were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure concentration was reported (24 hours) and appropriate for the study type.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was not explicitly reported. The study indicated that each chemical used in the study was tested at a minimum of 3 doses (appropriate for the study type). However, it was unclear how the mutagenic potency could be assessed "based on dose-response curves with at least three doses in the linear part of the curve" if only 3 doses were evaluated for some test substances. Each chemical was tested up to doses that induced toxicity (i.e., a rationale for dose selection). No information regarding concentration spacing was provided. Table 1 only provides one of the doses per strain, which was described as "a dose that produced a response at the highest point of the linear portion of the dose-response curve," (i.e. highest dose tested that did not produce cytotoxicity, which is considered to be an appropriate dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not used and not part of the study design (not absolutely required for studies of this type).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (S. typhimurium) is routinely used for the outcome of interest. Two of the strains used in the study (TA 1535 and TA 100) were obtained from a laboratory-maintained culture. Two additional strains (RSJ100 and TPT100) were generated by the study authors for these (and like) experiments. Information with respect to the genotype of the strains (markers) was provided.

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Study Citation:	D. M. Demarini, M. L. Shelton, S. H. Warren, T. M. Ross, J. Y. Shim, A. M. Richard, R. A. Pegram (1997). Glutathione S-transferase-mediated induction of GC->AT transitions by halomethanes in Salmonella Environmental and Molecular Mutagenesis, 30(4,4), 440-447
Data Type:	Mutagenicity assay
HERO ID:	657294

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per group appeared to be two, and was considered appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (mutation frequency up to doses that induced toxicity) addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups (exposure for 24 hours, with evaluations of revertant colonies after incubation for an additional 48 hours).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding 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	Low	× 1	3	Statistical analyses were not conducted. Summary data (mean, standard deviation, and sample size) were partially provided, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for this study type.
	Metric 23:	Data Interpretation	High	× 2	2	The study clearly specified the criteria for a positive response (at least a two-fold increase in the number of revertant colonies/plate relative to the control plates).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The study authors defined toxicity (thinning of the background lawn and/or a reduction in the number of revertants below that of controls). This assessment of toxicity is appropriate for the study type.
	Metric 25:	Reporting of Data	Low	× 2	6	Negative findings were reported qualitatively (i.e., TA1535 and TPT100 strains). For experiments with positive findings, data were not shown for each study group(but rather for one dose only).
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	D. M. Demarini, M. L. Shelton, S. H. Warren, T. M. Ross, J. Y. Shim, A. M. Richard, R. A. Pegram (1997). Glutathione S-transferase-mediated induction of GC->AT transitions by halomethanes in Salmonella Environmental and Molecular Mutagenesis, 30(4,4), 440-447
Data Type:	Mutagenicity assay
HERO ID:	657294

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 71: **In vitro evaluation results of Casanova et al 1997 for DPX and RNA adducts**

Study Citation:	M. Casanova, D. A. Bell, H. Heck (1997). Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes Fundamental and Applied Toxicology, 37(2,2), 168-180
Data Type:	DPX and RNA adducts for DCM
HERO ID:	730495

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]dichloromethane ([14C]DCM).
	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 purity of the test substance was reported (99%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	It did not appear that concurrent negative control groups were utilized in the study design. However, this is acceptable based on the study design (radiolabeled DNA binding).
	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 in detail and were appropriate for the endpoint of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation, handling, and storage of the test substance was described in detail and appropriate considering the volatility of the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent among treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in terms of mM DCM in the medium. Gas chromatography was performed to ensure the accuracy of the doses. Doses were not specified but could be estimated from Figures 1 and 3.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest (2 hours).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	M. Casanova, D. A. Bell, H. Heck (1997). Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes Fundamental and Applied Toxicology, 37(2,2), 168-180
Data Type:	DPX and RNA adducts for DCM
HERO ID:	730495

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	A variety of test models were utilized, including hepatocytes of male B6C3F1 mice, F344 rats, Syrian golden hamsters, and humans with and without functional GSTT1 genes. The identity, origin, isolation methods, and culture methods were described and appropriate for hepatocytes from all species. For the human hepatocytes, information such as demographics, health status, and method of isolation were provided.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition involving exposure to DCM included n = 2-3 replicates.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across all 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	Initial conditions were not reported for each group or replicate.
	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	Linear least squares regression analysis was used to analyze dose reposnse data for DNA-protein cross-links and RNA adducts.
	Metric 23:	Data Interpretation	High	× 2	2	The data were interpreted appropriately.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Viability was determined via trypan blue exclusion prior to and after the exposure period.
	Metric 25:	Reporting of Data	Medium	× 2	4	No measure of variance (i.e. standard deviation) was included.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	M. Casanova, D. A. Bell, H. Heck (1997). Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes Fundamental and Applied Toxicology, 37(2,2), 168-180
Data Type:	DPX and RNA adducts for DCM
HERO ID:	730495

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

Table 72: **In vitro evaluation results of Andrae and Wolff 1983 for in vitro DNA repair assay**

Study Citation:	U. Andrae, T. Wolff (1983). Dichloromethane is not genotoxic in isolated rat hepatocytes Archives of Toxicology, 52(4,4), 287-290					
Data Type:	in vitro DNA repair assay					
HERO ID:	730501					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as dichloromethane (DCM).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was identified
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reported (99.5%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A negative solvent (DMSO) control was included, however, it is uncertain whether the % DMSO used in the control was equivalent to the percentage used in the test groups.
	Metric 5:	Positive Controls	High	× 2	2	Methyl methanesulfonate and 2-acetylaminofluorene were used as positive controls and were valid inducers of DNA repair synthesis.
	Metric 6:	Assay Procedures	High	× 1	1	The assay procedures were 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	High	× 1	1	The preparation of the test solutions were described. The handling of the volatile test substance was well-described and appropriate. The storage of the test substance was not described, but this is appropriate given the study design (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	Concentration of the test substance applied and concentrations in the incubates 10 min after application were determined analytically by gas chromatography, indicating that approximately half of the applied dose volatilized into the gas phase of stoppered glass tubes.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (3hrs) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of dose groups and dose spacing was reported and appropriate. Previous studies were considered for dose selection.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	NA; study was performed in isolated rat hepatocytes which provide intracellular activation.

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Study Citation: U. Andrae, T. Wolff (1983). Dichloromethane is not genotoxic in isolated rat hepatocytes Archives of Toxicology, 52(4,4), 287-290
Data Type: in vitro DNA repair assay
HERO ID: 730501

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The source and isolation procedure for hepatocytes was described, however the origin of the animals used was not reported
	Metric 15:	Number per Group	Low	× 1	3	The number of cells used in the assay was appropriate. The results table reports two separate experiments. It appears that these are biological replicates, which n = 1 technical replicate per experimental condition per experiment (with the exception of n = 2 for the negative control, as indicated in the figure legend). This is considered to be lacking.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology was appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Test and control groups were consistently evaluated.
	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	The endpoint assessed was not subjective
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported in protocols across treatment groups
	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	Raw data are provided, but because n = 1 replicate for two experiments, statistical analysis is not possible.
	Metric 23:	Data Interpretation	Low	× 2	6	The evaluation criteria (radioactivity incorporated into light DNA) was an appropriate method for evaluating repair synthesis, however the criteria for determining positive outcomes was not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was not a direct measurement (doses were chosen to avoid cytotoxic effects), however the data indicates that the high dose resulted in a slight decrease in replicative synthesis.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
Overall Quality Determination‡			Unacceptable**		1.5	
Extracted			No			

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Study Citation:	U. Andrae, T. Wolff (1983). Dichloromethane is not genotoxic in isolated rat hepatocytes Archives of Toxicology, 52(4,4), 287-290
Data Type:	in vitro DNA repair assay
HERO ID:	730501

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 73: **In vitro evaluation results of Dillon et al 1992 for bacterial reverse mutation**

Study Citation:	D. Dillon, I. Edwards, R. Combes, M. Mcconville, E. Zeiger (1992). The role of glutathione in the bacterial mutagenicity of vapour phase dichloromethane Environmental and Molecular Mutagenesis, 20(3,3), 211-217					
Data Type:	Bacterial reverse mutation					
HERO ID:	730509					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified clearly by name (dichloromethane) and CASRN (75-09-2; the second dash was missing in the publication).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was specified; the test substance was obtained from a manufacturer (Rathburn Chemicals). 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 numerical purity of the test substance was not reported, but the test substance was reported to be HPLC grade. 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 negative controls (using air in place of DCM); it was specified that all conditions except exposure to the test substance were equal. Controls were set up concurrently with DCM exposure plates and were incubated for the same time.
	Metric 5:	Positive Controls	High	× 2	2	It was indicated that concurrent positive controls (MMS without S9 and 2-aminoanthracene with S9) were run with all experiments, and that there were no consistent differences with respect to the response of strains to the positive controls. No positive control data were shown; in multiple experiments, exposure to the test substance induced positive, exposure-related responses.
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures used in the experiments in sufficient detail (minor details were cited to other publications). The methods (e.g., using glass jars with lids for vapor exposures to DCM) were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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Study Citation:	D. Dillon, I. Edwards, R. Combes, M. Mcconville, E. Zeiger (1992). The role of glutathione in the bacterial mutagenicity of vapour phase dichloromethane Environmental and Molecular Mutagenesis, 20(3,3), 211-217
Data Type:	Bacterial reverse mutation
HERO ID:	730509

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Details on the preparation of the test substance were reported. It was indicated that exposure to DCM vapor was achieved by mixing the bacterial culture, metabolic activation mix or buffer, and top agar and pouring it onto plates. Plates were stacked in glass jars (known volume) with lids; quantities of DCM liquid necessary to give the desired concentrations were added to the jars. These methods appear to be appropriate (i.e., DCM as a volatile substance was prepared in sealed containers).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details regarding exposure administration were reported and were applied consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure durations (ranging from 2 to 48 hours) were reported, and were appropriate for the study type/outcome of interest. The study authors used various exposure durations to identify the optimum exposure time (6 hours).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and concentration spacing were considered adequate to address the purpose of the study. There were an adequate number of exposure groups (at least 4 plus controls); it was implied that the chemical was tested to concentrations that induced toxicity.
	Metric 13:	Metabolic Activation	High	× 1	1	The study authors reported that exposures were conducted in the presence and absence of metabolic activation. The types of actiavtion used were specified (S9, S100, microsomes, and liver homogenate) and the methods of preparation were described.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model(s) were reported (Salmonella typhimurium and Escherichia coli strains). The strains were obtained from laboratory-maintained cultures (Ames lab and/or National Collection of Industrial Bacteria) and are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per study group (triplicate) was reported and was appropriate for the study type.
Domain 5: Outcome Assessment						

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Study Citation:	D. Dillon, I. Edwards, R. Combes, M. Mcconville, E. Zeiger (1992). The role of glutathione in the bacterial mutagenicity of vapour phase dichloromethane Environmental and Molecular Mutagenesis, 20(3,3), 211-217
Data Type:	Bacterial reverse mutation
HERO ID:	730509

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (i.e., counting of revertant colonies) addressed the 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	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding 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	Statistical analyses were not performed (nor required); means plus SD were provided (enabling independent analyses).
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for determining a positive response were not explicitly specified.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity was defined by growth inhibition; this method is commonly used for assessments of this type. The study did not report levels of toxicity; these omissions are not likely to substantially impact the study results.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported consistently by exposure group (without omissions).
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 74: **In vitro evaluation results of Graves et al 1994 for DNA damage in mouse and rat hepatocytes**

Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA damage in mouse and rat hepatocytes: DCM and formaldehyde
HERO ID:	730537

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	Manufacturers were identified as the source.
	Metric 3:	Test Substance Purity	High	× 1	1	DCM was HPLC grade, formaldehyde was AnalaR grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A concurrent negative control was used, however; it is unclear whether it is an untreated or solvent control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric was not applicable to the outcome of interest.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were partially described and were cited to another publication, but appeared appropriate 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 of the test substance was reported, storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Volumes injected into the flask were increased with increasing dose.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in units of mM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Cells were exposed for 2h in vitro, which appeared appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	Three concentrations and a negative control were reported and were adequate to evaluate the outcome.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not needed for primary rodent hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was described and is appropriate for the study type.
	Metric 15:	Number per Group	High	× 1	1	Number cells per group in duplicate was reported an appeared adequate for the study.
Domain 5: Outcome Assessment						

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Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA damage in mouse and rat hepatocytes: DCM and formaldehyde
HERO ID:	730537

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive to the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent among exposure groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables 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	High	× 1	1	Means +/- SD values were provided graphically. An independent statistical analysis could be performed if the data were digitized.
	Metric 23:	Data Interpretation	High	× 2	2	Data criteria was reported and consistent with standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was reported in text as cell viability and was adequate for the study (methods were described).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes.
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 75: **In vitro evaluation results of Graves et al 1994 for DNA-protein crosslinks and DNA damage in Chinese hamster ovary cells**

Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA-protein crosslinks and DNA damage in CHO cells: DCM, DCM-GSH conj and formaldehyde
HERO ID:	730537

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	Manufacturers were reported as the source.
	Metric 3:	Test Substance Purity	High	× 1	1	HPLC grade for DCM, AnalaR grade for formaldehdye
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A concurrent negative control was used; however, it is unclear whether it is untreated or solvent control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods were partially described and were 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 study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported, storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Volumes injected into the flask were increased with increasing dose.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in units of mM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Cells were exposed for 2h in vitro, which appeared appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	3 concentrations and a negative control were reported and are adequate to evaluate the outcome.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Liver S9 were prepared from mice citing previous literature. It is a commonly used metabolic activation system.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was described and is appropriate for the study type.
	Metric 15:	Number per Group	High	× 1	1	The number of cells per group in duplicate was reported an appeared adequate for the study.

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Study Citation:	R. J. Graves, C. Coutts, H. Eyton-Jones, T. Green (1994). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice Carcinogenesis, 15(5,5), 991-996
Data Type:	DNA-protein crosslinks and DNA damage in CHO cells: DCM, DCM-GSH conj and formaldehyde
HERO ID:	730537

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive to the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent among exposure groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were 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	High	× 1	1	Data for DNA-proteing crosslinks were reported as mean +/- SD values which were sufficient for independent analysis. Alaline elution data were reported aws the mean of 2 duplicates; therefore, statistical analysis is not warranted.
	Metric 23:	Data Interpretation	High	× 2	2	Data criteria was reported and consistent with standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was reported in text as cell viability and the method was appropriate.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes
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 76: **In vitro evaluation results of Graves et al 1995 for in vitro DNA damage**

Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926
Data Type:	DNA damage in vitro
HERO ID:	730538

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as methylene chloride (MC).
	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, this is not expected to substantially impact the results given the short-term nature of the experiments.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were included in the study design.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. Although a positive control substance was not used, the study authors showed that a positive result could be induced in cell types used in the study (i.e., positive results for methylene chloride in lung cells and 1,2-dibromoethane in human cells).
	Metric 6:	Assay Procedures	Medium	× 1	2	Most assay procedures (e.g., temperatures, volumes, cell density) were described in adequate detail. Details with respect to the alkaline elution technique were cited to other publications.
	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	Test substance preparation methods were described; sealed containers were used to account for the volatility of the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups. It is noted that the study indicated that in Clara cells exposed at concentrations below 5 mM, MC was diluted in DMSO; however, Figure 5 shows data for exposures ranging from 5 to 60 mM only.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.

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Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926
Data Type:	DNA damage in vitro
HERO ID:	730538

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (1 to 2 hours) was reported. Similar studies typically utilize 3 to 6 hour exposures. This study used shorter exposure times; however, this was considered acceptable because the test substance (or other substances) gave a positive response.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate (e.g., at least 4 analyzable concentrations plus controls).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. Metabolic activation was used in an experiment in Chinese hamster ovary cells to elucidate the role of metabolism in MC-induced DNA damage; otherwise, primary cells were used.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were mouse lung (Clara) cells and hamster and human hepatocytes. Although not all of these cell types (with the exception of rodent hepatocytes or CHO cells) are typically used in genotoxicity experiments, they are considered adequate for the study type (nearly any eukaryotic cell type could be used). Information with respect to mice and hamsters were adequately described; however, data on human donors was sparse (e.g., number of donors specified, but sex and other demographic information was not provided).
	Metric 15:	Number per Group	High	× 1	1	The number of samples per group was reported and was considered appropriate for the endpoint of interest (range = 2 to 8 animals per species, with most experiments using two filters per alkaline elution measurement).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline elution measurement of single stranded breaks) was appropriate for the outcome of interest (DNA damage).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was reported to be consistent 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.
Domain 6: Confounding / Variable Control						

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Study Citation:	R. J. Graves, C. Coutts, T. Green (1995). Methylene chloride-induced DNA damage: An interspecies comparison Carcinogenesis, 16(8,8), 1919-1926
Data Type:	DNA damage in vitro
HERO ID:	730538

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data in Table 1 were analyzed using a Student's two-tailed t-test. Standard deviations could be estimated from mouse lung data (Figure 5) enabling independent statistical analyses.
	Metric 23:	Data Interpretation	High	× 2	2	Statistical analyses and/or a concentration-related increase in DNA breaks were considered a positive result. These criteria are considered consistent for studies of this type.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Methods for evaluating cytotoxicity were described (assessed by Trypan blue intake). Cytotoxicity endpoints were defined. The legend to Table 1 provides an indication when there was a greater than 2-fold increase in the number of cells permeable to trypan blue (at 90 to 120 mM).
	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 77: **In vitro evaluation results of Graves and Green 1996 for DNA damage and DNA-protein cross-links**

Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM DNA damage and DNA-protein cross-links
HERO ID:	730539

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was 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 test substance was reportedly HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were used (with and without activation).
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls were used (and not strictly required); however, test substances used in the study gave a positive response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were performed as previously described by Kohn et al. (1981) and Graves et al. (1994) for ss DNA breaks and Zhitkovitch and Costa (1992) for DNA-protein cross-links.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the volatile test substance was reported (sterile tubes for suspension method and/or sealed flasks for plate method). Storage conditions were not reported; this omission is not expected to substantially impact the study results owing to the short duration of the experiment.
	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 exposure concentration used was reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration (1 hour for suspension protocol) was reported. Although the typical exposure duration for studies of this type is 3 to 6 hours, this duration was chosen because a longer duration caused precipitation of the cytosolic components of the S100 fraction.

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Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM DNA damage and DNA-protein cross-links
HERO ID:	730539

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Only a single concentration of DCM was tested, however, the purpose of the study was to evaluate mutagenicity in relation to metabolism (rather than the dose response). Therefore, the single DCM dose (in conjunction with increasing concentrations of liver cytosol) were appropriate to address the outcome of interest.
	Metric 13:	Metabolic Activation	High	× 1	1	Details of the metabolic activation system were adequately reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (CHO cell) origin and maintenance conditions were reported. This test model is routinely used in genotoxicity assays.
	Metric 15:	Number per Group	Low	× 1	3	Only a single replicate was tested; this may substantially impact the study results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	DNA damage was assessed by measuring the amount of DNA retained on filters (considered appropriate to evaluate the outcome of interest). Parts of the assessment methodology was cited to other publications.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Test and control groups were consistently evaluated.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	It was indicated that inter-experimental differences may be due to differences in the quality of metabolic activation preparations.
	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 were not performed (as only 1 replicate was used). However, data were presented graphically for independent analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not clearly specified.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cell survival was for the concentrations tested was reported as part of a different experiment in the same study (not concurrently).

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Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM DNA damage and DNA-protein cross-links
HERO ID:	730539

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Medium	× 2	4	Data were reported adequately (suspension assay). However, it is not clear if this aspect of the study was also performed using the plate assay. The study results state that increased DNA breaks were not seen "when cells were exposed to higher concentrations of MC as attached cultures " (i.e., the plate protocol).
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 78: **In vitro evaluation results of Graves and Green 1996 for mutagenicity in Chinese hamster ovary cells**

Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM mutagenicity in CHO cells
HERO ID:	730539

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was 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 test substance was reportedly HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were used (with and without metabolic activation).
	Metric 5:	Positive Controls	Medium	× 2	4	1,2-DBE was used as a reference chemical class-specific genotoxin (plate assay); however, it is unclear if this was used concurrently with MC because data were reported separately. Although no positive control was used in the suspension assay, the test substance was shown to induce mutations in the presence of activation (type of control recommended for this study type).
	Metric 6:	Assay Procedures	High	× 1	1	The assays procedures were well-described. Mutagenicity was evaluated using plate and suspension protocols with activation with or without GSH.
	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	High	× 1	1	The media test solution components were reported. The study indicated that cells were exposed to the volatile test substance in tightly capped flasks. Although storage conditions were not reported, this omission is not expected to substantially impact the results (owing to the short duration of the experiments).
	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 (0.5% for the plate protocol and 0.3% v/v for the suspension protocol) were reported without ambiguity.

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Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM mutagenicity in CHO cells
HERO ID:	730539

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure durations (4 hours for the plate protocol and 1 hour for the suspension protocol) were reported. Although the typical exposure time for studies of this type is 3 to 6 hours, exposure for 1 hour (suspension protocol only) was chosen because longer exposures caused there to be a noticeable precipitation of cytoslic components when activation was used.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Only a single concentration of DCM was tested in the plate and suspension assays; however, the purpose of the study was to evaluate mutagenicity in response to metabolism (rather than to evaluate the dose-response). Therefore, the dose used (in conjunction with increasing concentrations of metabolic activation) were appropriate to address the purpose of the study. The study used a dose of the test substance that did not cause cytotoxicity.
	Metric 13:	Metabolic Activation	High	× 1	1	Information about the metabolic activation system was adequately described.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (CHO cell) origin and maintenance conditions were reported. This cell type is routinely used to evaluate the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cultures used was appropriate. In general, assays using DCM were performed in duplicate or triplicate; in a few cases, single treated cultures were used (e.g., the GSH depletion experiment). Given that experiments only varied in % metabolic activation or GSH content (not DCM concentration), this is not expected to impact the study results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology was standard for the time of the study and appropriate for the endpoint of interest (i.e., 8 days for expression of the mutant phenotype consistent with the recommendation for studies of this type).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Test and control groups were consistently evaluated.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	R. J. Graves, T. Green (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay Mutation Research: Genetic Toxicology, 367(3,3), 143-150
Data Type:	DCM mutagenicity in CHO cells
HERO ID:	730539

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study authors indicated that inter-experimental differences may have resulted from differences in the quality of the metabolic activation preparations.
	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	High	× 1	1	Appropriate statistical analysis was performed and and fold increases were reported in the text.
	Metric 23:	Data Interpretation	High	× 2	2	Statistical significance and/or about a two-fold increase in mutation frequency was considered the indicator for a positive response.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cell survival was included in the study. Endpoints were defined and described adequately.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
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 79: **In vitro evaluation results of Kayser and Vuilleumier 2001 for DNA damage**

Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA damage
HERO ID:	730547

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 (dichloromethane).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was specified; the test substance was obtained from a manufacturer (Sigma). 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/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative control groups were used (e.g., minimal medium with methanol without the addition of DCM) for the experiment (e.g., to evaluate DNA damage in wild-type and DNA-repair deficient strains).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately and were appropriate for the endpoint of interest. Methods were described mainly in the figure legends; there was no methods section of the paper.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance was adequately described. Although storage conditions were not reported, this omission is not likely to impact the study results given the duration of the study (12 hours).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentration of DCM used in the experiment was reported without ambiguity (10 mM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest (12 hours; as evidenced by observable differences in the accumulation of DNA damage).

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Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA damage
HERO ID:	730547

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Only one concentration of DCM was utilized; DNA damage was not evaluated in a time- or exposure-related manner. However, this is considered to be adequate given the study design and positive results observed at this dose.
	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	Medium	× 2	4	The test model, Methylobacterium dichloromethancium (wild-type and DNA repair-deficient) appeared to be appropriate for the outcome of interest (i.e., methylotrophic bacteria to evaluate DNA damage owing to growth with DCM). However, few details about the strains (e.g., the source) were provided.
	Metric 15:	Number per Group	Medium	× 1	2	It does not appear that more than one experiment was conducted. It is inferred from the error bars on Figure 3A that replicates were utilized, but it is not clear how many.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate. The study stated that increased labeling was indicative of DNA strand breaks.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across all treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in the study design were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables in outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	It does not appear that statistical analyses were conducted. Means and variance could be estimated from Figure 3 to enable independent statistical analysis (not explicitly specified that bars represent standard errors or standard deviations). However, the number of replicates is not specified, so independent statistical analysis is not possible.

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Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA damage
HERO ID:	730547

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	The data were interpreted appropriately (i.e., radiolabeled DNA as the indicator of a positive response). However, the threshold for a positive response was not specified by the study authors.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study authors did not explicitly define cytotoxicity parameters; however, it is indicated that growth was monitored (e.g., when evaluating DCM dehalogenase levels). The limited details with respect to cytotoxicity endpoints is not expected to substantially impact the study results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Number of replicates was not reported. Measure of variance (standard deviation or SEM) was not specified.
Overall Quality Determination‡			Unacceptable⋆⋆		1.4	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 80: **In vitro evaluation results of Kayser and Vuilleumier 2001 for DNA adducts**

Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA adducts
HERO ID:	730547

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 (dichloromethane).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was specified; the test substance was obtained from a manufacturer (Sigma). 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/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative control groups were used (e.g., absence of DCM dehalogenase or GSH in the 14C-labeled DCM experiment; absence of DCM in the 35S-labeled GSH experiment) for the experiment (e.g., to evaluate GST-mediated DNA adduct formation). For these controls, all conditions appeared to be equal except for the addition of a specific component of the reaction mixture.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately and were appropriate for the endpoint of interest. Methods were described mainly in the figure legends; there was no methods section of the paper.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance was adequately described. Although storage conditions were not reported, this omission is not likely to impact the study results given the duration of the study (60 min).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentration of DCM used in the experiment was reported without ambiguity (50 mM).

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Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA adducts
HERO ID:	730547

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest (60 min; as evidenced by observable DNA adduct formation).
	Metric 12:	Exposure Route and Method	High	× 1	1	Only one concentration of DCM was utilized; DNA damage was not evaluated in a time- or exposure-related manner. However, this is considered to be adequate given the study design and positive results observed at this dose.
	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	The test model, calf thymus DNA, was reported and appropriate for the outcome of interest. Limited details were provided, but this is unlikely to have a substantial impact on results, as the calf thymus DNA was obtained from a commercial source.
	Metric 15:	Number per Group	Medium	× 1	2	The production of DNA adducts via GST-mediated conversion of DCM was evaluated in at least two independent experiments. One experiment utilized labeled DCM and the second experiment utilized labeled GSH.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate; the base specificity of DNA adduct formation was subsequently evaluated in the study report.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across all treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in the study design were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables in outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	It does not appear that statistical analyses were conducted. Means and variance could be estimated from Figure 1 to enable independent statistical analysis (not explicitly specified that bars represent standard errors or standard deviations).

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Study Citation:	M. F. Kayser, S. Vuilleumier (2001). Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts Journal of Bacteriology, 183(17,17), 5209-5212
Data Type:	DNA adducts
HERO ID:	730547

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	The data were interpreted appropriately (i.e., radiolabeled DNA as the indicator of a positive response). However, the threshold for a positive response was not specified by the study authors.
	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	Number of replicates was not reported. Measure of variance (standard deviation or SEM) was not specified.
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 81: **In vitro evaluation results of Landi et al 2003 for DNA damage**

Study Citation:	S. Landi, A. Naccarati, M. K. Ross, N. M. Hanley, L. Dailey, R. B. Devlin, M. Vasquez, R. A. Pegram, D. M. DeMarini (2003). Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 538(1-2,1-2), 41-50
Data Type:	DNA damage
HERO ID:	730553

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified (dichloromethane; CH2Cl2).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was specified; the test substance was obtained from a manufacturer (Aldrich). 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 such that any observed effects were highly likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using negative controls; however, it is not clear whether controls were treated with DMSO vehicle.
	Metric 5:	Positive Controls	Low	× 2	6	DCM was included as a positive control for GSTT1-1 activation (considered a chemical class-related reference substance); however, the expected response was not observed (i.e., there was not a significant increase in DNA damage in cells from GSTT1-1+ donors). Induction of DNA damage (albeit weak) was observed in cells from some subjects after exposure to DCM and other chemicals.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were described in adequate detail (e.g., composition of media, cell density, passage details, electrophoresis, pH, slide preparation).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was adequately described (DCM was prepared in DMSO when cells reached confluence). Although storage conditions were not reported, this omission is not likely to impact the study results given the short duration of the study (3 hours).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were consistent across study groups.

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Study Citation:	S. Landi, A. Naccarati, M. K. Ross, N. M. Hanley, L. Dailey, R. B. Devlin, M. Vasquez, R. A. Pegram, D. M. DeMarini (2003). Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 538(1-2,1-2), 41-50
Data Type:	DNA damage
HERO ID:	730553

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity (10, 100, 1000 uM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (3 hours) was consistent with standards for studies of this type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study used 3 analyzable concentrations of the test substance; this is standard for studies of this type. A rationale for the concentrations used was not specified; however, it was indicated that there have been previous genotoxicity studies using these chemicals, and that the highest dose induced toxicity.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The cell type (primary human lung epithelial cells) is not routinely used/cells used typically in genotoxicity tests were not used. The source of the cells (4 human subjects) was reported. The cell type was selected based on its relevance to (inhalation) exposure and because the cell type normally expresses GSTT1-1.
	Metric 15:	Number per Group	High	× 1	1	It appeared that the number of replicates per group was appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology (measured as tail extent moment) did not fully address the outcome of interest (DNA damage). Deficiencies in the study (inadequate enzymatic activity, effects of freezing/culturing the cells) resulted in poor sensitivity of the assay. Therefore, it was indicated that the study was useful only for evaluating the baseline response to genotoxicity.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	At least 100 nuclei per concentration were scored for tail extent moment; 100 cells (50 cells per replicate slide) were analyzed. The number of cells/slides evaluated were appropriate for the outcome of interest.

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Study Citation:	S. Landi, A. Naccarati, M. K. Ross, N. M. Hanley, L. Dailey, R. B. Devlin, M. Vasquez, R. A. Pegram, D. M. DeMarini (2003). Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 538(1-2,1-2), 41-50
Data Type:	DNA damage
HERO ID:	730553

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	High	× 1	1	The study indicates that the four donors were coded in the comet assay (as A-D).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were differences in the tissues used that likely impacted the stusy results (i.e., considerable inter-individual variation).
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	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 used in the study report were adequately described, and statistical significance was clearly presented in the data tables.
	Metric 23:	Data Interpretation	High	× 2	2	The study authors reported the criteria for a positive response (statistical analyses were used, and the exposure-relatedness of the effect was considered).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The methods used for evaluating toxicity were adequately described; however, data related to cytotoxicity were not shown, and cytotoxicity at the highest dose was relatively high (about 50%) compared to standards for studies of this type (around 30%).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			~~High~~ ⟶ 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 =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

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

§ Evaluator's explanation for rating change: "Owing to deficiencies in the study design/execution, the data presented in the study are useful for evaluating only the baseline response to genotoxicity (no adequate positive control/activation system was used)."

Table 82: **In vitro evaluation results of Marsch et al 2004 for DNA adducts**

Study Citation:	G. A. Marsch, S. Botta, M. V. Martin, W. A. Mccormick, F. P. Guengerich (2004). Formation and mass spectrometric analysis of DNA and nucleoside adducts by S-(1-acetoxymethyl)glutathione and by glutathione S-transferase-mediated activation of dihalomethanes Chemical Research in Toxicology, 17(1,1), 45-54
Data Type:	DNA adducts for DCM
HERO ID:	730567

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 CH2Cl2 (DCM).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of DCM 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	Concurrent negative controls (test system excluding DCM or excluding other components) were included in the study design.
	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 and were appropriate for the endpoint of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation and handling of the test substance was described in detail and appropriate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent among treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported adequately.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (30 min) was appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	High	× 1	1	Several different GST sytems were utilized (bacterial and human).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model, calf thymus DNA, was reported and appropriate for the outcome of interest. Limited details were provided, but this is unlikely to have a substantial impact on results, as the calf thymus DNA was obtained from a commercial source.

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Study Citation:	G. A. Marsch, S. Botta, M. V. Martin, W. A. Mccormick, F. P. Guengerich (2004). Formation and mass spectrometric analysis of DNA and nucleoside adducts by S-(1-acetoxymethyl)glutathione and by glutathione S-transferase-mediated activation of dihalomethanes Chemical Research in Toxicology, 17(1,1), 45-54
Data Type:	DNA adducts for DCM
HERO ID:	730567

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Only one sample per treatment group was analyzed. However, this is considered to be adequate considering the outcome of interest. It was noted that "because it was necessary to compare several different sets of experiments within the same time frame, the number of reactions that we could assess by HPLC-MS was limited." Therefore, this metric, and others relating to number per group (i.e. statistical analysis) are not applicable to the study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across all 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	Medium	× 2	4	The concentrations of the original GST enzyme preparations from human, rat, and bacteria were different; there, it was not possible to use the same concentrations in the experiments. The authors accounted for this by normalizing data from each enzyme preparation to that of the bacterial GST concentration. No other confounding variables related to exposure were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 23:	Data Interpretation	High	× 2	2	The data were interpreted appropriately.
	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	High	× 2	2	All data were reported adequately.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	G. A. Marsch, S. Botta, M. V. Martin, W. A. Mccormick, F. P. Guengerich (2004). Formation and mass spectrometric analysis of DNA and nucleoside adducts by S-(1-acetoxymethyl)glutathione and by glutathione S-transferase-mediated activation of dihalomethanes Chemical Research in Toxicology, 17(1,1), 45-54
Data Type:	DNA adducts for DCM
HERO ID:	730567

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 83: **In vitro evaluation results of Hu et al 2006 for DNA damage**

Study Citation:	Y. Hu, S. L. Kabler, A. H. Tennant, A. J. Townsend, A. D. Kligerman (2006). Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 607(2,2), 231-239
Data Type:	DNA damage
HERO ID:	730573

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances (DCM and formaldehyde, a metabolite) were clearly identified both by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substances was specified; the test substance was obtained from a manufacturer (Sigma). Although batch/lot numbers were not provided, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity/grade of the test substances was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative control groups were used (solvent controls); all conditions except exposure to the test substances were equal.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the responses for DCM and formaldehyde were positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures used in the test were described in adequate detail and were 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	It was indicated that DCM was dissolved in DMSO and formaldehyde was dissolved in phosphate-buffered saline. Although storage conditions were not reported, this omission is not likely to impact the study results given the short duration of the study (2 hours).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were consistently administered across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity (0, 2.5, 5, and 10 mM for DCM; 0, 150, 300, and 600 uM for formaldehyde).

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Study Citation:	Y. Hu, S. L. Kabler, A. H. Tennant, A. J. Townsend, A. D. Kligerman (2006). Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 607(2,2), 231-239
Data Type:	DNA damage
HERO ID:	730573

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure varied slightly from the standard (2 hours, rather than 3 to 6 hours); however, the duration of the study enabled the detection of effects (and therefore, was considered adequate for the study type).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study used three analyzable concentrations of the test substances. Based on data presented in the study report, concentrations used in the study induced toxicity at the highest levels (cell viability decreased by about 16% to 58% for DCM and 15% to 18% for formaldehyde at the highest exposure concentration dependent on assay used). Although no rationale for dose selection was provided, the concentrations used were adequate to generate exposure-related responses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. The study used a cell line with the mouse construct for GSTT1; the expression of GSST1 was tested for its ability to activate DCM (with respect to cytotoxicity and genotoxicity).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model used was appropriate for the outcome of interest. V79 cells are commonly used for genotoxicity assays, and the cell line was obtained from a specified source (a laboratory-maintained strain that originally came from the MRC Cell Mutation Unit in England). This parent cell line was used to generate the other cell line used in the study, mGSST1 cells. The study authors performed experiments to validate that V79 mGSST1 cells expressed GSST1.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that data shown were the mean for three experiments. The number of replicates per study group was considered appropriate for the study type.
Domain 5: Outcome Assessment						

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Study Citation:	Y. Hu, S. L. Kabler, A. H. Tennant, A. J. Townsend, A. D. Kligerman (2006). Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 607(2,2), 231-239
Data Type:	DNA damage
HERO ID:	730573

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcome of interest and was particularly sensitive for the outcome of interest. It was indicated that the modified technique used in the study (i.e., use of proteases) allowed the detection of DNA damage caused by agents that induce cross-linking (e.g., formaldehyde).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment were reported and were applied consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study indicated that 50 cells per slide and two slides per treatment were evaluated (100 cell total per group). The number of cells/slides was evaluated was considered appropriate for the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not mentioned in the study report.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial conditions were not reported for each group or replicate.. The study authors indicated that the use of two cells lines that only differed with respect to the expression of the gene of interest (GSST1) reduced confounding associated with using cells with different genetic backgrounds.
	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	Data manipulation and statistical methods were described in adequate detail. The study indicated that data shown represented means +/- SEM for replicate independent experiments. The types of statistical tests used were specified (e.g., ANOVA, Dunnett's).
	Metric 23:	Data Interpretation	High	× 2	2	The study authors reported the criteria for a positive response (statistical analyses were used, and the exposure-relatedness of the effect was considered).

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Study Citation:	Y. Hu, S. L. Kabler, A. H. Tennant, A. J. Townsend, A. D. Kligerman (2006). Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 607(2,2), 231-239
Data Type:	DNA damage
HERO ID:	730573

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	High	× 1	1	Cytototoxicity was evalulated using three different assays (trypan blue assay, live/dead cytotoxicity assay, neutral red assay). The methods and procedures used to perform these assays were described in detail. In general (and depending on the assay used), cytotoxicity was within a range that is considered standard for studies of this type (< 30%). Data from the cytotoxicity assays were shown in full in the study report.
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 84: **In vitro evaluation results of Pegram et al 1997 for bacterial reverse mutation**

Study Citation:	R. A. Pegram, M. E. Andersen, S. H. Warren, T. M. Ross, L. D. Claxton (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in Salmonella typhimurium: contrasting results with bromodichloromethane off chloroform Toxicology and Applied Pharmacology, 144(1,1), 183-188
Data Type:	Bacterial reverse mutation
HERO ID:	730581

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 (methylene chloride).
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from a manufacturer (EM Science). Although a lot/batch number was nto provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99.99%). The purity of the test substance was such that any observed effects are highly likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Traditional positive controls were not used (not absolutely required). However, the response for DCM (and other chemicals) was positive and/or exposure-related. DCM was used in the study to provide a basis for comparison of mutagenic potency relative to trihalomethanes.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially cited to other publications (i.e., Maron and Ames 1983 for standard plate-incorporation mutagenicity and modifications for testing volatile chemicals according to Hughes et al. 1987). Modifications of the Hughes et al. 1987 protocol were described (e.g., injection of chemical vapor rather than liquid).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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Study Citation:	R. A. Pegram, M. E. Andersen, S. H. Warren, T. M. Ross, L. D. Claxton (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in Salmonella typhimurium: contrasting results with bromodichloromethane off chloroform Toxicology and Applied Pharmacology, 144(1,1), 183-188
Data Type:	Bacterial reverse mutation
HERO ID:	730581

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The test substance prepartion conditions were reported. It was indicated that standard and stock concentrations were prepared by injecting measured amounts of the test substance into Tedlar bags fitted with injection ports and filled with appropriate volumes of sterile air. Bags were heated to volatilize the chemicals and kept at room temperature in darkness or under yellow light after preparation. It appears that appropriate steps were taken to account for the volatility of the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were consistently administered across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity. It was indicated that target concentrations were 0, 200, 400, 800, and 1600 ppm. GC analysis for dosage determination indicated that these target concentrations produced 0, 0.03, 0.06, 0.13. and 0.26 mM DCM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure concentration was reported (24 hours) and appropriate for the study type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were minor limitations with respect to the number/spacing of exposure groups; no rationale was provided (other than the possible clue that the highest exposure concentration was toxic to the bacteria). Four analyzable concentrations of the test substance were utilized in the study.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	No exogenous activation system was used. However, the study used a strain with the rat construct for GST; the expression of GST T1 was tested for its ability to activate DCM (with respect to genotoxicity).
Domain 4: Test Model						

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Study Citation:	R. A. Pegram, M. E. Andersen, S. H. Warren, T. M. Ross, L. D. Claxton (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in Salmonella typhimurium: contrasting results with bromodichloromethane off chloroform Toxicology and Applied Pharmacology, 144(1,1), 183-188
Data Type:	Bacterial reverse mutation
HERO ID:	730581

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The test model (Salmonella typhimurium) is routinely used in bacterial reverse mutation assays. S. typhimurium TA 1535 was obtained from a laboratory-maintained culture (Ames lab). The other two strains used in this study were TA 1535 -GST (with GST cDNA inserted in the opposite [nonfunctional] direction) and TA 1535 +GST (strain transfected with rat GSH S-transferase) were obtained from another laboratory (Dr. Guengerich at Vanderbilt University School of Medicine). It was previously demonstrated that TA 1535 -GST showed negligible GST activity, and that the TA 1535 +GST strain showed GST T1-1 expression (Thier et al., 1993). It was indicated that the vector used to transform the +GST strain contained an ampicillin resistance marker.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per study group were appropriate for the study type and analysis. The figure legend (Figure 1) states that the data represent means +/- standard deviations from a minimum of 4 plates per concentration.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (revertants/plate) addressed the intended outcome of interest, and appeared to be sensitive to the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups (exposure for 24 hours, with evaluations of revertant colonies after incubation for an additional 48 hours).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for groups or replicates.
	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						

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Study Citation:	R. A. Pegram, M. E. Andersen, S. H. Warren, T. M. Ross, L. D. Claxton (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in Salmonella typhimurium: contrasting results with bromodichloromethane off chloroform Toxicology and Applied Pharmacology, 144(1,1), 183-188
Data Type:	Bacterial reverse mutation
HERO ID:	730581

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were described briefly. The study stated that data were analyzed using one-way ANOVA to determine if mutations induced by the test substance were significantly greater than the spontaneous mutations in a given strain. The data figure (Figure 1) also provides (graphically) means and standard deviations that could be used for independent analyses.
	Metric 23:	Data Interpretation	Medium	× 2	4	The study partially addresses the criteria for a positive response (with a heavy reliance on statistical significance). The study does not explicitly address biological relevance (which is typically considered first for studies of this type). Given that a significantly increased response was observed over the concentration range tested (except the highest exposure level), this is not expected to substantially impact the study results.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were not fully defined or reported. The study indicated that the highest exposure concentration of DCM was toxic to the bacteria (there were not other mentions of cytotoxicity measurements). Although the remaining doses used in the study showed significantly increased numbers of revertants relative to controls, the effect was not strictly concentration-related (i.e., the second highest exposure concentration did not have the highest number of revertants); it is unclear if toxicity impacted these results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported for most outcomes by exposure group. However, it appears that chemicals were also tested in S. typhmurium TA 1535, and no data for that strain were shown or discussed qualitatively.
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

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Study Citation:	R. A. Pegram, M. E. Andersen, S. H. Warren, T. M. Ross, L. D. Claxton (1997). Glutathione S-transferase-mediated mutagenicity of trihalomethanes in Salmonella typhimurium: contrasting results with bromodichloromethane off chloroform Toxicology and Applied Pharmacology, 144(1,1), 183-188
Data Type:	Bacterial reverse mutation
HERO ID:	730581

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 85: **In vitro evaluation results of Zielenska et al 1993 for mutagenicity**

Study Citation:	M. Zielenska, A. Ahmed, M. Pienkowska, M. Anderson, B. W. Glickman (1993). Mutational specificities of environmental carcinogens in the lacI gene of Escherichia coli. VI: Analysis of methylene chloride-induced mutational distribution in Uvr+ and UvrB- strains Carcinogenesis, 14(5,5), 789-794
Data Type:	Mutagenicity
HERO ID:	732107

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 (DCM or methylene chloride).
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from a manufacturer (Sigma). Although a lot/batch number was nto provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity/grade of the test substances was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	It was indicated (indirectly) that a negative control group was used, but details regarding the negative control group were not reported. The study indicated that treatment with DCM produced an increase "over the spontaneous lacI- mutation frequency." The spontaneous mutation frequencies of E. coli strains were reported in the legend of Table II.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Traditional positive controls were not used (not absolutely required). The response for DCM was positive, suggesting that the assay could effectively detect mutations.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods/procedures were partially described, and partially cited to other sources (Zielenska et al. 1989). Some details such as culture media and incubation temperature were reported, however, other details such as cell density were not specified (only indicated that cells were grown to mid-lag phase).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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Study Citation: M. Zielenska, A. Ahmed, M. Pienkowska, M. Anderson, B. W. Glickman (1993). Mutational specificities of environmental carcinogens in the lacI gene of Escherichia coli. VI: Analysis of methylene chloride-induced mutational distribution in Uvr+ and UvrB- strains Carcinogenesis, 14(5,5), 789-794
Data Type: Mutagenicity
HERO ID: 732107

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported in minimal detail (treatment with DCM occurred in glass culture flasks). It is not entirely clear how well the volatility of the test substance was accounted for in handling procedures. Although storage conditions were not reported, this omission is not likely to impact the study results given the short duration of the study (30 minutes).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details on exposure administration were inferred from the text; however, omissions are not likely to substantially impact the study results.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentration used was reported without ambiguity (2% DCM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure to the test substance was for 30 minutes, but was sufficient to induce mutation. The plates were incubated for 72 hours (standard for studies of this type).
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	There were deficiencies in the number of exposure groups for this bacterial reverse mutation assay (two bacterial strains exposed to 0 or 2% DCM). Given that a mutagenic response was elicited and the outcome of interest was regarding the frequencies of different types of mutations (i.e. base substitution, frameshift, deletion, etc) in different bacterial strains, this is not considered to have substantially impacted the results. However, the one dose chosen is not reliable. A "survival level" of 32% and 18% was reported for Uvr+ and UvrB-, respectively. The significant cytotoxicity at the single dose of DCM used renders the results irrelevant.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	No exogenous activation system was used (or required by study type).
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported without additional information. It was indicated that two strains of Escherichia coli were used, uvr+ (excision repair-proficient), and Uvr- (excision repair-deficient). The source of the strains was not specified.

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Study Citation: M. Zielenska, A. Ahmed, M. Pienkowska, M. Anderson, B. W. Glickman (1993). Mutational specificities of environmental carcinogens in the lacI gene of Escherichia coli. VI: Analysis of methylene chloride-induced mutational distribution in Uvr+ and UvrB- strains Carcinogenesis, 14(5,5), 789-794
Data Type: Mutagenicity
HERO ID: 732107

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Although it wasn't explicitly specified, it appears that the number of replicates per group was appropriate for the study type. It was not explicitly specified how many replicate plates there were, but it is inferred from the text that replicate plates were used because the study states five independenbt cultures of each strain were used, and that mutants were selected from P-gal plates (plural). The number of mutants selected (400 Uvr+ and 700 Uvr-) also suggest that multiple plates were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Based on the observation that the mutation frequency was 6-fold (wild-type Uvr+) and 8-fold higher (Uvr-) than spontaneous levels in DCM-treated cells, the outcome assessment appeared to be sensitive to the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups (exposure for 30, with incubation of plates for an additional 72 hours).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding 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	Unacceptable	× 1	4	Statistical analyses of revertant colonies were not conducted, and sufficient data were not provided to enable independent statistical analyses. As revertants/plate were also not provided, it is also not possible to draw conclusions based on fold-changes, which is acceptable for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not reported and omissions are likely to substantially impact the study results.

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Study Citation:	M. Zielenska, A. Ahmed, M. Pienkowska, M. Anderson, B. W. Glickman (1993). Mutational specificities of environmental carcinogens in the lacI gene of Escherichia coli. VI: Analysis of methylene chloride-induced mutational distribution in Uvr+ and UvrB- strains Carcinogenesis, 14(5,5), 789-794
Data Type:	Mutagenicity
HERO ID:	732107

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Evaluations of cytotoxicity were performed, but the methods of measurement were not described. The study only indicated that the "survival level" was 32% for the wild-type Uvr+ strain and 18% in the Uvr- strain.
	Metric 25:	Reporting of Data		× 2	NA	Data presentation with respect to the mutagenicity portion of this study was inadequate. The numbers of revertants/plate were not shown for any exposure group. The focus of the study was the characterization of specific mutants obtained from DCM-treated cells.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 86: **In vitro evaluation results of Olvera-Bello et al 2010 for sister chromatid exchange in human peripheral blood mononuclear cells**

Study Citation:	A. E. Olvera-Bello, E. Estrada-Muñiz, G. Elizondo, L. Vega (2010). Susceptibility to the cytogenetic effects of dichloromethane is related to the glutathione S-transferase theta phenotype Toxicology Letters, 199(3,3), 218-224
Data Type:	Sister chromatid exchange (SCE) in human PBMCs - DCM
HERO ID:	783479

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as dichloromethane (75-09-2).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (i.e., manufacturer) was reported. The product number and batch/lot number were not reported. Given that the test substance is not expected to vary in composition, this omission is not likely to impact the study results.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors report using an appropriate negative control group .
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design (and not strictly required). Although a positive control was not used, the test substance gave a positive, dose-related response (indicative of effective assay conditions).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were mostly described; some assay procedures were cited to a previously published study (Gonsebatt et al. 1992).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was described (prepared freshly each time). The study authors verified that DCM was stable in culture conditions (using gas chromatography).
	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	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (72 hours). Based on the study results, the duration of exposure appeared relevant to detect the outcome of interest.

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Study Citation:	A. E. Olvera-Bello, E. Estrada-Muñiz, G. Elizondo, L. Vega (2010). Susceptibility to the cytogenetic effects of dichloromethane is related to the glutathione S-transferase theta phenotype Toxicology Letters, 199(3,3), 218-224
Data Type:	Sister chromatid exchange (SCE) in human PBMCs - DCM
HERO ID:	783479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Six concentrations plus control were used; concentrations were based on established permissible exposure limits (Mexican NOM-010-STPS-1999).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. The study evaluated the role of activation by GST enzymes on the DNA-damaging effects of DCM.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and source were reported. Details regarding human donors were provided (age, sex, smoking status, etc). This test model (peripheral blood cells) is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of tissues per study group was reported and appropriate for the study type (4 low-, 10 medium- and 6 high GSTT1 activity individuals).
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	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling for the outcome of interest was adequate (for SCEs, 25 consecutive second-division metaphases with 46 centromeres were scored).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables were identified. The study controlled for GSTT1 activity, a variable that would/did have had an impact on the study 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	High	× 1	1	Sister chromatid exchanges (SCEs) were statistically analyzed across groups. The analyses appeared appropriate to the study type.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate (e.g., statistically significantly increased numbers of SCEs).

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Study Citation:	A. E. Olvera-Bello, E. Estrada-Muñiz, G. Elizondo, L. Vega (2010). Susceptibility to the cytogenetic effects of dichloromethane is related to the glutathione S-transferase theta phenotype Toxicology Letters, 199(3,3), 218-224
Data Type:	Sister chromatid exchange (SCE) in human PBMCs - DCM
HERO ID:	783479

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	High	× 1	1	The study evaluated cytotoxicity (measured as mitotic index) as well as cytostaticity (measured as cell proliferation kinetics). The endpoints were well-defined and methods of measurement were described.
	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 87: **Animal toxicity evaluation results for 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 DCM
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 dichloromethane (DCM).
	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 contrary to the guideline of at least two daily 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	Low	× 1	3	Only one dose of DCM was utilized.
	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						

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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 DCM
HERO ID:	38908

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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.
	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). Guidelines standards suggest 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 were reported for this experiment.
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		1.6	
Extracted			Yes			

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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 DCM
HERO ID:	38908

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: **In vitro evaluation results of Olvera-Bello et al 2010 for sister chromatid exchange in human peripheral blood mononuclear cells**

Study Citation:	S. Mimaki, Y. Totsuka, Y. Suzuki, C. Nakai, M. Goto, M. Kojima, H. Arakawa, S. Takemura, S. Tanaka, S. Marubashi, M. Kinoshita, T. Matsuda, T. Shibata, H. Nakagama, A. Ochiai, S. Kubo, S. Nakamori, H. Esumi, K. Tsuchihara (2016). Hypermutation and unique mutational signatures of occupational cholangiocarcinoma in printing workers exposed to haloalkanes Carcinogenesis, 37(8,8), 817-826
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	3419931

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 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 (99.5%) was such that effects were likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study reported using a concurrent negative control (presumably filter paper without added DCM, but not explicitly specified).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type (and not strictly required). However, treatment-related positive responses were observed (i.e., the test is capable of detecting a positive response).
	Metric 6:	Assay Procedures	Medium	× 1	2	The study briefly described modifications to the standard plate-incorporation method. Methods/procedures were partially cited to another publication (DeMarini et al. 1997).
	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	Vapor generation methods were described in limited detail. The study indicates that doses of DCM were applied to appropriately sized filter papers; plates were placed into tightly sealed bags so that bacteria were exposed to the evaporating test substance. The study indicated that modifications were made to the standard plate-incorporation protocol owing to the volatility of the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	S. Mimaki, Y. Totsuka, Y. Suzuki, C. Nakai, M. Goto, M. Kojima, H. Arakawa, S. Takemura, S. Tanaka, S. Marubashi, M. Kinoshita, T. Matsuda, T. Shibata, H. Nakagama, A. Ochiai, S. Kubo, S. Nakamori, H. Esumi, K. Tsuchihara (2016). Hypermutation and unique mutational signatures of occupational cholangiocarcinoma in printing workers exposed to haloalkanes Carcinogenesis, 37(8,8), 817-826
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	3419931

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were not explicitly reported, but could be estimated from graphical information (Figure 2). The high-dose was specified in the text (3500 ppm). Chemical vapor concentrations were determined by gas chromatography mass spectrometry analysis.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure (2 hours) was reported and appeared to be appropriate for the study type (increased numbers of revertants were seen post-exposure).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups (3 groups plus controls) was reported (fewer than recommended number). A rationale was not provided for concentration spacing, but doses were adequate to elicit a dose-response.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. The study aimed to compare the mutational signatures among workers exposed occupationally to DCM (and other solvents) and bacteria exposed to DCM.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with minimal descriptive information (hisG marker as indicator of reversion). The test model (Salmonella typhimurium strain TA 100) is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The study indicates that duplicate plates were used (at least two independent experiments).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported, and was considered appropriate for the outcome of interest (quantification of revertant colonies as an indicator of mutagenicity after 2 hours exposure/48 hours incubation).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It appeared that outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	S. Mimaki, Y. Totsuka, Y. Suzuki, C. Nakai, M. Goto, M. Kojima, H. Arakawa, S. Takemura, S. Tanaka, S. Marubashi, M. Kinoshita, T. Matsuda, T. Shibata, H. Nakagama, A. Ochiai, S. Kubo, S. Nakamori, H. Esumi, K. Tsuchihara (2016). Hypermutation and unique mutational signatures of occupational cholangiocarcinoma in printing workers exposed to haloalkanes Carcinogenesis, 37(8,8), 817-826
Data Type:	Bacterial reverse mutation for DCM
HERO ID:	3419931

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported, but are not expected to impact the study results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not conducted (for the number of revertants at the his locus), and estimations of variance were not provided for all dose groups, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response was inferred from the text. The study indicates that the test substance showed mutagenicity based on a dose-related increased number of revertants.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	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	Data were reported by exposure group.
Overall Quality Determination‡			High		1.4	
Extracted			No			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 89: **In vitro evaluation results of Yang et al 2014 for DNA damage**

Study Citation:	F. Yang, J. Zhang, W. Chu, D. Yin, M. R. Templeton (2014). Haloactamides versus halomethanes formation and toxicity in chloraminated drinking water Journal of Hazardous Materials, 274 156-163
Data Type:	DNA damage
HERO ID:	3493441

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 (dichloromethane; DCM).
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from a manufacturer. Although a lot/batch number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substances used in the study were at least analytical grade; therefore, observed effects are very 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 control. There was reference to a concurrent negative control group in the notes accompanying Table 2, a DMSO group (presumably vehicle-only control group) was shown in Figure 3, and the text corresponding to this table and figure mentions non-treated cells (as a control). It is inferred that all conditions except exposure to the test substance were equal among groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the responses for DCM (and other chemicals used in the study) were positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Medium	× 1	2	Nearly all of the assay methods and procedures were cited to the Supplementary Material (https://www.sciencedirect.com/science/article/abs/pii/S0304389414002702 It was indicated that the methods and procedures used were similar to those used in previous assays of the same type (the only difference being the cell line used).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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Study Citation:	F. Yang, J. Zhang, W. Chu, D. Yin, M. R. Templeton (2014). Haloactamides versus halomethanes formation and toxicity in chloraminated drinking water Journal of Hazardous Materials, 274 156-163
Data Type:	DNA damage
HERO ID:	3493441

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	No details were provided with respect to test substance preparation. Based on the data provided, the test substance appeared to be diluted in DMSO. Although storage conditions were also not reported, the acute nature of the experiment suggests that this omission is not likely to substantially impact the study results.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	It is inferred from the text that exposures were administered consistently across study groups; however, most information pertaining to procedures/methods were cited to the Supplementary Material.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity (Figure 3).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure is not clearly cited in the study report. However, the study states that the assay was conducted in a similar manner as previous assays (the only difference being the cell type used). In addition, detailed information regarding procedures and methods were cited to the Supplementaty Material.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and concentration spacing were adequate. In the absence of observed cytotoxicity, DCM was tested at concentrations as high as 5000 mg/L; 5 analyzable DCM concentrations were used.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	No exogenous activation system was used (or required by study type).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited descriptive information; this cell type is not typically used in studies of this type. The cell line was obtained from an appropriate source (Cell Bank of the Chinese Academy of Sciences).
	Metric 15:	Number per Group	Medium	× 1	2	The number of replicates per group was not explicitly specified in the study report; however, data were shown in Figure 3 with a measure of variation (suggesting replicate experiments), and other in vitro assays conducted as part of the same study used at least triplicate samples. Methods/procedures specific to the single cell gel eelectrophoresis (SCGE) assay were cited to the Supplementary Material.

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Study Citation:	F. Yang, J. Zhang, W. Chu, D. Yin, M. R. Templeton (2014). Haloactamides versus halomethanes formation and toxicity in chloraminated drinking water Journal of Hazardous Materials, 274 156-163
Data Type:	DNA damage
HERO ID:	3493441

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (measurement of tail moment) was appropriate for and sensitive to the outcome of interest (DNA damage).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	It is inferred from the text that outcomes were assessed consistently across study groups; however, most information pertaining to procedures/methods were cited to the Supplementary Material.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number cells/slides evaluated was not explicitly specified in the study report. However, the study states that the assay was conducted in a similar manner as previous assays (the only difference being the cell type used). Methods/procedures specific to the single cell gel eelectrophoresis (SCGE) assay were cited to the Supplementary Material.
	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	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 analyses pertaining to this particular assay were not described in detail (Table 2 indicative of ANOVA); however, this omission is unlikely to substantially affect the study results. In addition, data shown in Figure 3 enable independent/statistical analyses.
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response could be inferred from the text. Statistical analyses were performed. In addition, the text accompanying Figure 3 addresses the concentration-relatedness of the effect.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The study authors defined cytotoxicity endpoints (cytotoxicity was assessed using the MTT assay). Although methods and procedures were largely cited to the Supplementary Material, cytotoxicity data were shown in the study report (Figure 2).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.6	

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Study Citation:	F. Yang, J. Zhang, W. Chu, D. Yin, M. R. Templeton (2014). Haloactamides versus halomethanes formation and toxicity in chloraminated drinking water Journal of Hazardous Materials, 274 156-163
Data Type:	DNA damage
HERO ID:	3493441

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 90: **Animal toxicity evaluation results of Narotsky et al 1995 for an oral developmental study (gestation day 6-19) on reproductive, growth (early life) and development, neurological/behavioral, respiratory, body weight, and mortality**

Study Citation:	Narotsky, MG; Kavlock, RJ (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity Journal of Toxicology and Environmental Health, 45(2), 145-171
Data Type:	Oral developmental study (GD 6-19)
HERO ID:	76052

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Dichloromethane (99.9%)
	Metric 2:	Test Substance Source	Medium	× 1	2	Aldrich Chemical Co.; batch no. not reported
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control (corn oil)
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not needed for study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Placed in group using nonbiased procedure that assured a homogenous distribution of body weights among groups. Control for BW introduces nonrandom component.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Mixed with corn oil for gavage. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent across groups; gavage volume of 1 ml/kg
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	0, 337.5, 450 mg/kg-d
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	GD 6-19 -Current guidance suggests that organogenesis is from day 5 in rodents, but even suggests that dosing can start even earlier to obtain rets of pre-implanatation etc.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure groups plus control; exposures don't cover a wide range of doses either and thus, not clear whether a dose-response relationship can be demonstrated.
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage in corn oil
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Timed-pregnant F344 rats (~90-d-old). Initial BW 150-225g. Obtained from Harlan Sprague Dawley Inc.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Consistent across groups; reported adequately.

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Study Citation: Narotsky, MG; Kavlock, RJ (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity Journal of Toxicology and Environmental Health, 45(2), 145-171
Data Type: Oral developmental study (GD 6-19)
HERO ID: 76052

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Medium	× 1	2	16-21/group; OECD TG 414 suggests a least 20 pregnant dams per group; thus, lower numbers/group are more for screening purposes.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Maternal toxicity: survival, clinical signs, body weight (GD 6, 8, 10, 13, 16, 20) Repro/dev't: resorptions, implants, # live litters, live pups on PND 1 and PND 6, pup weight, gross pup examination; any dead pups were examined for gross malformations and soft-tissue alterations. Usual developmental toxicity studies look at visceral, skeletal and external malformations; this is more of screening level study.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were assessed for relevant outcomes
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for examined endpoints.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported; no deviations from expected noted.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Groups had homogeneous distribution of BW at study initiation. Other confounding variables not identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	2 deaths (one in each exposure group) attributed to gavage error but not likely to influence results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Dams with one implant excluded from statistical analysis. Pup examination data were not statistically analyzed (considered anecdotal). Other data analyzed using General Linear Models (GLM) procedure.
	Metric 24:	Reporting of Data	Medium	× 2	4	Maternal toxicity: Quantitative data for mortality and BW (reported graphically), clinical signs reported qualitatively only Repro/Dev't: Quantitative data for most outcomes (reported graphically or in tables); gross examination of pups reported qualitatively only
Overall Quality Determination[‡]			High		1.4	
Extracted			Yes			

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Study Citation:	Narotsky, MG; Kavlock, RJ (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity Journal of Toxicology and Environmental Health, 45(2), 145-171
Data Type:	Oral developmental study (GD 6-19)
HERO ID:	76052

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 91: **Animal toxicity evaluation results of General et al 1976 for a combined 1-generation and subchronic oral toxicity study in rats on reproductive, growth (early life) and development, hematological and immune, neurological/behavior, renal, hepatic, ocular and sensory, cardiovascular, endocrine, clinical chemistry/biochemical, endocrine, gastrointestinal, mortality, musculoskeletal/motor function, body weight, respiratory, and thyroid outcomes**

Study Citation:	GE (1976). Dichloromethane: Reproduction and ninety day oral toxicity study in rats
Data Type:	Combined 1-gen and subchronic oral toxicity study in rats
HERO ID:	730464

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Dichloromethane
	Metric 2:	Test Substance Source	Medium	× 1	2	The compound was-received from the General Electric Company, Mount Vernon, Indiana on December 10, 1975. The compound was a clear liquid and was identified as "Dichloromethane* Reagent, A.C.S. CH2C12 FW 84.94 DX835 5509 Matheson Coleman & Bell Manufacturing Chemists". But the study has the following comment: The above description is not totally accurate. The compound was furnished to IR&DC in containers labeled as indicated above but the actual contents were not from the indicated source. The contents were withdrawn on 12/4/75 from a purchased railroad tank -car of methylene chloride purchased from Dow Chemical certified to meet GE plastics Incoming Material Specification PCM-l-Sl. This methylene chloride is typical of that being used currently to produce Lexan® polycarbonate resin in the Mt. Vernon plant.
	Metric 3:	Test Substance Purity	Low	× 1	3	Not reported; study authors state "This methylene chloride is typical of that being used currently to produce Lexan® polycarbonate resin in the Mt. Vernon plant."
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control group administered distilled water via gavage on the same regimen as treated rats.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for this type of study
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						

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Study Citation:	GE (1976). Dichloromethane: Reproduction and ninety day oral toxicity study in rats
Data Type:	Combined 1-gen and subchronic oral toxicity study in rats
HERO ID:	730464

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The compound was dissolved in distilled water at a concentration of 15 mg/ml for gavage administration. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volume differed between groups (15 ml/kg-d for 0 and 225 mg/kg-d; 1.67 ml/kg-d for 25 mg/kg-day; 5.0 ml/kg-d for 75 mg/kg-d). The vehicle is distilled water so this difference should not significantly impact results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	0, 25, 75, or 225 mg/kg-d via gavage
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Total exposure: F0 rats 18 weeks; F1 rats 13 weeks. Methods section did not specifically state how long F0 rats were exposed prior to mating, but exposure ended at weaning. Based on Tables 5 and 6 (food consumption in F0 animals), weeks 11-13 were mating. So, F0 rats were exposed 10 weeks prior to mating, for 3 weeks during mating, and through gestation and lactation. It is not stated explicitly in the methods whether the 90-d exposure in F1 rats included 3 wks of nursing or not. Again, based on F1 food consumption table (Table 7) for F1 rats, it appears that the 13-wk F1 exposure was post-weaning (13 wks of F1 food consumption data)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Based on lack of effects at highest dose, this may not have been a high enough exposure to inform toxicity of DCM. The only exposure-related finding reported was a slight, transient decrease in pup body weight on PND 21 at 75 mg/k-d (8%) and 225 mg/kg-d (15%). At study week 0 (assuming post-weaning), F1 body weights at these doses did not differ from control.
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Charles River CD rats, 71-101 g
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions consistent. House individually (except during mating and lactation periods) in wire cages; temperature and humidity controlled room. Food and water available ad libitum. Temp and humidity not reported.

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Study Citation:	GE (1976). Dichloromethane: Reproduction and ninety day oral toxicity study in rats
Data Type:	Combined 1-gen and subchronic oral toxicity study in rats
HERO ID:	730464

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Medium	× 1	2	F0: 10/sex/group; F1: 15/sex/group; For a reproductive toxicity study (OECD TG 415), there should be enough animals for the result to be 20 pregnant animals/group. Using 10 animals/group is more of a screening reproductive toxicity study (e.g., OECD TG 421).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Histopathology on a large number of organs/tissues, as well as hematology, biochemistry, urinalysis, body weight, clinical signs were taken.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation.
	Metric 18:	Sampling Adequacy	High	× 1	1	F0 10/group; F1 15/group (1o F1 controls and 10 F1 high-dose for histo; low- and mid-dose groups not evaluated due to lack of high-dose effects - consistent with protocol)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Study endpoints do not require blinding.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses reported; no deviations from standard reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting BW reported; body weight effects only reported in F1 rats on PND 21, and were minimal.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical tests reported for reproductive and developmental endpoints. Statistics not reported for non-reproductive/dev't endpoints; data reporting for survival and body weight adequate for independent statistics. Other endpoints inadequate for statistics (qualitative)
	Metric 24:	Reporting of Data	High	× 2	2	Mortality, Bd wt data, food consumption, and repro/dev't data reported quantitatively. Other endpoints (no exposure-related effects) reported qualitatively. Note that tables of all effects are included in appendices
Overall Quality Determination[‡]			High		1.5	
Extracted			Yes			

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Study Citation:	GE (1976). Dichloromethane: Reproduction and ninety day oral toxicity study in rats
Data Type:	Combined 1-gen and subchronic oral toxicity study in rats
HERO ID:	730464

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 92: **Animal toxicity evaluation results of Raje et al 1988 for inhalation study on reproductive outcomes**

Study Citation:	Raje, R., Basso, M., Tolen, T., Greening, M. (1988). Evaluation of in vivo mutagenicity of low-dose methylene chloride in mice International Journal of Toxicology, 7(5,5), 699-703
Data Type:	Reproduction inhalation study
HERO ID:	732088

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified definitively by chemical name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer was reported without batch/lot no.
	Metric 3:	Test Substance Purity	Medium	× 1	2	HPLC grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Air exposed control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups. Only males were exposed.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target concentrations and actual concentrations (mean +-SD) were reported.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure was for only 2h/day (5 days/wk, 6 weeks)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	There were 3 exposure groups, but the levels were narrowly spaced. (100, 150 and 200 ppm). It is unclear whether the highest dose was high enough. No justification was provided for levels.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic whole body chamber, vapor may condense; airchanges not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Females were not exposed prior to or during mating and gestation.
	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	20 males/group
Domain 5: Outcome Assessment						

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Study Citation:	Raje, R., Basso, M., Tolen, T., Greening, M. (1988). Evaluation of in vivo mutagenicity of low-dose methylene chloride in mice International Journal of Toxicology, 7(5,5), 699-703
Data Type:	Reproduction inhalation study
HERO ID:	732088

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Limited number of parameters were evaluated, including testes histopathology, pregnancy index and uterine examination data.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were measured consistently across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Litter data was provided.
	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 parameters were objective).
	Metric 20:	Negative Control Response	High	× 1	1	Responded as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported and DCM is expected 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	Low	× 1	3	Statistics were not described; however, text indicate that no statistically significant changes were found.
	Metric 24:	Reporting of Data	Low	× 2	6	# Post-implantation deaths were not directly reported (reported as % dead/litter). Pre-implantation loss could not be determined because corpora lutea were not measured.
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