United States
Environmental Protection Agency

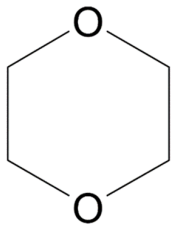
Office of Chemical Safety and
Pollution Prevention

Risk Evaluation for 1,4-Dioxane

Systematic Review Supplemental File:

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

CASRN: 123-91-1



December 2020

EPA's Office of Pollution Prevention and Toxics (OPPT) developed data quality criteria for animal and in vitro studies, presented in the *Application of Systematic Review in TSCA Risk Evaluations* document (EPA Document #740-P1-8001). This document presents data quality evaluation results for animal and in vitro studies evaluated for the 1,4-Dioxane Risk Evaluation.

Table Listing

Acute (<24 hr)

Short-term (1-30 days)

Subchronic (30-90 days)

Chronic (>90 days)

Developmental

Genetic toxicity studies

Mechanistic

1 Acute (<24 hr)

Table 1: **Animal toxicity evaluation results of Drew et al 1978 for a 4-hour inhalation study on clinical chemistry/biochemical outcomes (hepatic enzymes)**

Study Citation:	Drew, RT; Patel, JM; Lin, FN (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819
Data Type:	4-hour inhalation
HERO ID:	67913

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).
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported and a batch/lot number was not provided; however, the report states that substances were purchased from conventional sources and were assayed for purity by gas chromatography.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A concurrent negative control group was tested, but was not described in detail (e.g., number per group, treatment method) to allow a determination of whether it was appropriate and comparable to the treated groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control group is not necessary for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study did not completely report the method and equipment used to generate the test substance atmosphere; however, there was no reason to believe that there was an impact on animal exposure. Information on storage was not reported; however, there was no reason to suggest that the test substance was unstable.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure were reported for the most part and there was no indication to suggest that the exposures differed among the groups.

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Study Citation: Drew, RT; Patel, JM; Lin, FN (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819
Data Type: 4-hour inhalation
HERO ID: 67913

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentrations were reported as nominal values. Vapor test concentrations were monitored continuously by an automatic gas sampling gas chromatograph; however, actual concentrations were not reported. Due to the lack of reporting of actual concentrations for vapor exposures, I downgraded this metric to low.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration and frequency were reported (4 hours, one exposure) and suitable for the study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing (1000 and 2000) ppm were relevant for the assessment.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The route of exposure (inhalation) was reported and was suited to the test substance. The method of exposure was not specifically stated. Additionally, the number of air changes per hour was not reported, so I downgraded the score to low.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex and starting body weight were reported; however, age and health status at the start of the study were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions (temperature, humidity, light cycle) were not sufficiently reported to evaluate if husbandry was adequate and similar among the groups, so I downgraded the score for this metric to low.
	Metric 15:	Number per Group	Medium	× 1	2	The exact number of animals per group was not reported. The authors stated that each experiment started with 15 animals, , The authors stated that consecutive daily heart punctures, which were performed to collect blood for serum enzyme assay analyses, resulted in several deaths, but the exact number of deaths, or final number of animals/blood samples collected per group, was not reported. Nevertheless, the results appear to have been sufficient for statistical analysis, so I scored this metric as medium.
Domain 5: Outcome Assessment						

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Study Citation:	Drew, RT; Patel, JM; Lin, FN (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819
Data Type:	4-hour inhalation
HERO ID:	67913

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology for this acute exposure study was limited to clinical chemistry/biochemistry parameters, specifically, serum enzyme analysis.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology appeared to be consistent among the groups in terms of the procedures used to measure the different serum enzymes. There was no indication that methods differed between groups for timing of blood collection for analysis.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and acceptable for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective endpoints were evaluated in this study.
	Metric 20:	Negative Control Response	High	× 1	1	Each rat served as its own control prior to exposure.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding differences reported among the study groups; however, initial body weight or food/water intake were not reported. Additionally, respiratory rate was not reported, but 1,4-dioxane is a potential respiratory irritant, so I downgraded the score to low.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only differences among groups for the evaluated outcomes were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described in sufficient detail and were appropriate for the data sets.
	Metric 24:	Reporting of Data	Low	× 2	6	Data presentation is incomplete. No data were presented for control groups.
Overall Quality Determination‡			Medium		2.2	
Extracted			Yes			

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Study Citation:	Drew, RT; Patel, JM; Lin, FN (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819
Data Type:	4-hour inhalation
HERO ID:	67913

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 2: **Animal toxicity evaluation results of Uno et al 1994 for an acute oral study on mechanistic (gene expression/omics, genotoxicity) outcomes**

Study Citation:	Uno, Y; Takasawa, H; Miyagawa, M; Inoue, Y; Murata, T; Yoshikawa, K (1994). An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens Mutation Research, 320(3), 189-205
Data Type:	Acute oral
HERO ID:	194385

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported (Tokyo Chem Industry Co). A batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A concurrent negative/vehicle control group was tested but it appears that results for the control were only based on T = 0, rather than a true control, which was sampled at each time point (i.e., also 24, 39, 48 hours post-treatment/administration of vehicle, i.e., see Table 1).
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance was dissolved or suspended in corn oil; however, no other details were provided on test substance preparation or storage methods.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure were reported and there was no indication to suggest that the exposures differed among the groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The administered doses (1000 and 2000 mg/kg via gavage) were reported. It appears that these were per body weight doses, although not specifically stated.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported (single exposure with evaluation at up to 48 hours post-exposure.. These appear acceptable for the intended outcomes for the study (mechanistic).

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Study Citation: Uno, Y; Takasawa, H; Miyagawa, M; Inoue, Y; Murata, T; Yoshikawa, K (1994). An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens Mutation Research, 320(3), 189-205

Data Type: Acute oral

HERO ID: 194385

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 and were justified by the study authors (were based on the MTD).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were reported and were considered appropriate for the purpose of the study.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, age, sex, and source were reported; however, body weight and health status at the start of the study were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions (temperature and light) were reported and were similar for all groups. Humidity was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number per group (n = 4) was smaller than is typical for a study of this type (acute exposure) but was appropriate for the intended outcomes and purpose of the study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was reported and was sensitive for the outcomes of interest although it's not clear that the duration (up to 48 hours post-exposure) was sufficient to address the intended outcomes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology appeared to be consistent among the groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling methods appear to have been appropriate for addressing the outcomes of interest (2000 hepatocytes/liver (n = 4)) were evaluated for replicative DNA synthesis (RDS). It's not clear, however, how cell viability was determined (i.e., how many cells were sampled).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were evaluated in this study.
	Metric 20:	Negative Control Response	High	× 1	1	Biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No confounding variables in test design were reported; however, initial body weight and food/water intake were not reported.

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Study Citation:	Uno, Y; Takasawa, H; Miyagawa, M; Inoue, Y; Murata, T; Yoshikawa, K (1994). An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens Mutation Research, 320(3), 189-205
Data Type:	Acute oral
HERO ID:	194385

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only differences among groups for the evaluated outcomes were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and were appropriate for the data sets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented (RDS incidence and cell viability, only mechanistic outcomes were reported).
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 3: **Animal toxicity evaluation results of Mattie et al 2012 for a 6-hour inhalation study on neurological/behavioral outcomes**

Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	6-hour inhalation study - neuro
HERO ID:	3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)
	Metric 3:	Test Substance Purity	High	× 1	1	>99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were exposed to clean air. 2 separate control groups were used to ensure concurrent exposure group for all 5 exposure levels (only 4 total exposure chambers).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for study type (OPPTS 870.1300)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were "randomly selected for each exposure group".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Vapor generation method was adequately reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were consistent between groups. In the low-dose group (target 100 ppm), there was a problem in the air handling system of the chamber, resulting in a large spike in concentration during the first hour. The issue was resolved, but resulted in a large standard deviation.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target, nominal, and analytical concentrations reported (Table 3). Exposure chamber concentrations were continuously sampled and the concentration determined approximately every 40 seconds by FTIR analysis for each entire 6 hour exposure.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration consistent with cited guideline (OPPTS 870.1300)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Five exposure groups plus concurrent controls were used. Exposure levels were based on levels in previous studies.

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Study Citation: Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type: 6-hour inhalation study - neuro
HERO ID: 3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic, whole-body exposure with 15 complete fresh air changes per hour; individually housed in 690 L chambers. Any aerosols that were formed during vaporization process were captured by a patch of glass wool upstream, so nose-only exposure was not necessary.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Albino inbred Fischer (CDF®) [F344/DuCrl] rats. Age not reported. Based on weights (150-200g for males, 125-175g for females) they were young adults.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.
	Metric 15:	Number per Group	High	× 1	1	10/sex/group; 5/sex sacrificed two days after start of exposure, 5/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group observed for 14 days)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Clinical signs of neurotoxicity (autonomic effects, central nervous system effects, and reactivity to handling or sensory stimuli)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessment identical across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling consisted with cited guideline (OPPTS 870.1300)
	Metric 19:	Blinding of Assessors	Unacceptable	× 1	4	No reporting of blinding status of examiners during subjective assessments of clinical signs of neurotoxicity.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	Results of clinical signs evaluations not reported for control or exposure group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Methods section states that evaluations of respiration were conducted, but respiratory rate was not reported (no reporting of clinical signs, or lack thereof). Rated as low since 1,4-dioxane is a respiratory irritant.

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Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	6-hour inhalation study - neuro
HERO ID:	3563367

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	No mortalities were reported. Minimal serous exudate and few acute and chronic leukocyte infiltrates that were observed in a small number of rats distributed across all groups, controls and treated, were attributed to "environment irritants and/or a mild resolving bacterial infection"; observed at both 2 day and 14 day sacrifice. This is not expected to impact neurological assessment.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	Unacceptable	× 1	4	No mention of statistical analysis of clinical neurotoxicity evaluation (data not reported).
	Metric 24: Reporting of Data	Unacceptable	× 2	8	Results of clinical signs evaluations not reported for control or exposure group.
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 4: **Animal toxicity evaluation results of Mattie et al 2012 for a 6-hour inhalation systemic effects study on hepatic, renal, irritation, respiratory, body weight outcomes**

Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	6-hour inhalation study - systemic effects
HERO ID:	3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)
	Metric 3:	Test Substance Purity	High	× 1	1	>99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were exposed to clean air. 2 separate control groups were used to ensure concurrent exposure group for all 5 exposure levels (only 4 total exposure chambers).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for study type (OPPTS 870.1300)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were "randomly selected for each exposure group".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Vapor generation method was adequately reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were consistent between groups. In the low-dose group (target 100 ppm), there was a problem in the air handling system of the chamber, resulting in a large spike in concentration during the first hour. The issue was resolved but resulted in a large standard deviation.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target, nominal, and analytical concentrations reported (Table 3). Exposure chamber concentrations were continuously sampled and the concentration determined approximately every 40 seconds by FTIR analysis for each entire 6 hour exposure.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration consistent with cited guideline (OPPTS 870.1300)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Five exposure groups plus concurrent controls were used. Exposure levels were based on levels in previous studies.

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Study Citation: Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type: 6-hour inhalation study - systemic effects
HERO ID: 3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic, whole-body exposure with 15 complete fresh air changes per hour; individually housed in 690 L chambers. Any aerosols that were formed during vaporization process were captured by a patch of glass wool upstream, so nose-only exposure was not necessary.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Albino inbred Fischer (CDF®) [F344/DuCrl] rats. Age not reported. Based on weights (150-200g for males, 125-175g for females) they were young adults.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.
	Metric 15:	Number per Group	High	× 1	1	10/sex/group; 5/sex sacrificed two days after start of exposure, 5/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group observed for 14 days)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Hepatic, Renal - OW, HP Respiratory - HP of entire respiratory tract, including nasal sections Body weight - at randomization, prior to exposure, weekly during post-exposure, and at necropsy
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessment identical across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling consisted with cited guideline (OPPTS 870.1300)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only non-subjective outcomes and initial histopathological evaluations performed; blinding not necessary.
	Metric 20:	Negative Control Response	Medium	× 1	2	Control histopathological data were not explicitly stated, but based on qualitative statements regarding what was found in higher exposure groups, it is inferred that lesions were not observed in controls. Qualitative statement regarding no statistically significant changes in organ weight or body weight covers both control and exposure groups.
Domain 6: Confounding / Variable Control						

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Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	6-hour inhalation study - systemic effects
HERO ID:	3563367

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Methods section states that evaluations of respiration were conducted, but respiratory rate was not reported (no reporting of clinical signs, or lack thereof). Rated as low since 1,4-dioxane is a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No mortalities were reported. Minimal serous exudate and few acute and chronic leukocyte infiltrates that were observed in a small number of rats distributed across all groups, controls and treated, were attributed to "environment irritants and/or a mild resolving bacterial infection"; observed at both 2 day and 14 day sacrifice.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	BW and OW data analyzed by t-test and ANOVA. No statistical analysis of lesion incidence. Exposure-related nasal lesion incidence is reported in higher exposure groups - if it is assumed that lesion incidence is 0/5 for groups without explicitly reported lesions, statistical analysis could be conducted . Incidental findings that were observed in "all groups" were reported qualitatively only (not adequate for statistical analysis).
	Metric 24:	Reporting of Data	Medium	× 2	4	BW/OW - Qualitative (no effects) Histo - Exposure-related nasal lesion incidence is reported in higher exposure groups (assumed 0/5 for other groups, but not explicitly reported). Incidental findings that were observed in "all groups" were reported qualitatively only.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~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

§ Evaluator's explanation for rating change: "Due to some limitations in data reporting (requiring reader to make inferences) and study author's indication that other environmental irritants or infection may have been present, the study was downgraded to medium. However, since nasal lesions were observed at high exposure levels (in addition to the nasal irritation findings in all groups), the study still appears adequate to identify exposure-related findings."

Table 5: **Animal toxicity evaluation results of Dow et al 1989 for a single dose in vivo DNA synthesis study on hepatic, genotoxicity, body weight outcomes**

Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Single dose in vivo DNA synthesis
HERO ID:	4158030

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,4-dioxane
	Metric 2:	Test Substance Source	Medium	× 1	2	Baker Chemical Company; no batch number, but purity was analyzed by study laboratory
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle (saline) control was used
	Metric 5:	Positive Controls	Unacceptable	× 1	4	No positive control; in vivo genotoxicity study design indicates one should have been used (DMN was used in the repeat dose study only)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were computer randomized into treatment groups in all experiments
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Storage details not reported. Mixed with saline for gavage administration.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions consistent between groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Replicate 1: 0, 100, or 1000 mg/kg Replicate 2: 0, 10, 100, or 1000 mg/kg
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Once, sacrificed after 7 d
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2-3 doses plus negative control (two replicates)
	Metric 12:	Exposure Route and Method	Medium	× 1	2	No rationale was provided for switching from gavage (this study) to repeat-dose drinking water study (accompanying study). Other compounds (HCBD, DMN) were administered via gavage for both studies. However, BWG was decreased by ~45-55% following single gavage administration of 1000 mg/kg; this BW effect was not observed with drinking water administration of 1000 mg/kg over 11 weeks. SO perhaps the change in route was due to the decreased body weight associated with gavage administration.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Male SD rats (Spartan Research). Based on weight (180-260g), they were adult animals.

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Study Citation: Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type: Single dose in vivo DNA synthesis
HERO ID: 4158030

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry was consistent between groups (wire cages, environmentally controlled rooms, food and water ad libitum). Number of rats/cage was not reported, environmental conditions not reported.
	Metric 15:	Number per Group	High	× 1	1	4/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Genotox, organ weight, and histology of liver (cancer target organ); body weight and food consumption also monitored.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	4/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only non-subjective and initial histological evaluations; blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	negative control response was reported; no deviations from normal were reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Unacceptable	× 2	8	Initial BW 180-260 g (not reported per group). Body weight gains decreased 45-55% at 1000 mg/kg and 33-40% at 10-100 mg/kg. Decreased food consumption (magnitude not reported) associated with decreased BW. This may be the reason that drinking water route was used for repeat-dose study (same high exposure dose level).
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Weight loss may have been due to exposure route (bolus exposure) as opposed to (or in addition to) toxic effects. No weight effects observed at the same exposure level in accompanying repeated exposure drinking water study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Continuous data were compared by Dunnett's t-test. No statistical analysis of histopathological findings. Histological findings only reported qualitatively.
	Metric 24:	Reporting of Data	Medium	× 2	4	DNA synthesis, liver weight, and BWG reported quantitatively with statistics. Histopathological findings reported qualitatively (present or absent at dose).
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

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Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Single dose in vivo DNA synthesis
HERO ID:	4158030

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

2 Short-term (1-30 days)

Table 6: **Animal toxicity evaluation results of Goldberg et al 1964 for a 10-day inhalation study on neurological/behavior, body weight outcomes**

Study Citation:	Goldberg, ME; Johnson, HE; Pozzani, UC; Smyth, HF, Jr (1964). Effect of repeated inhalation of vapors of industrial solvents on animal behavior: I. Evaluation of nine solvent vapors on pole-climb performance in rats American Industrial Hygiene Association Journal, 25(4), 369-375
Data Type:	10-day inhalation
HERO ID:	58035

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified definitively.
	Metric 2:	Test Substance Source	Low	× 1	3	The report states that chemicals were obtained commercially; however, source or analytical verification of test substance were not reported. No batch/lot numbers were reported. The omitted details are not likely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was tested and was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control group is not necessary for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomized and distributed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Methods and equipment used for generating the test atmospheres were reported; however, storage conditions for the test substance were not reported, so I downgraded the score for this metric to medium.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Actual concentrations were not reported. Concentrations were reported as nominal values. Vapor test concentrations were monitored during the exposures and air flows were adjusted so that the actual vapor concentrations were within 10% of nominal concentrations. Due to the lack of reporting of actual concentrations for vapor exposures, I downgraded this metric to low.

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Study Citation:	Goldberg, ME; Johnson, HE; Pozzani, UC; Smyth, HF, Jr (1964). Effect of repeated inhalation of vapors of industrial solvents on animal behavior: I. Evaluation of nine solvent vapors on pole-climb performance in rats American Industrial Hygiene Association Journal, 25(4), 369-375
Data Type:	10-day inhalation
HERO ID:	58035

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 were 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 dose/concentration spacing were adequate to address the purpose of the study. Selected concentrations were not justified by the study authors but the range of concentrations was appropriate.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The route of exposure (inhalation) was reported and was suited to the test substance. The method of exposure was not specifically stated, but appears to have been dynamic whole-body exposure, based on the study methods description, and is considered suitable for the test substance. The number of air changes per hour was not reported, so I downgraded the score to low.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, age, and starting body weight were reported. Health status at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions (temperature, humidity, light cycle) were not sufficiently reported to evaluate if husbandry was adequate and similar among the groups, so I downgraded the score for this metric to low.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group (8/group) was lower than the typical number used in repeated-dose studies, but sufficient for statistical analysis and this minor limitation is unlikely to have a substantial impact on results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was reported and specific for the outcomes of interest (neurobehavioral effects). However, the study did not include a post-mortem examination of neural tissue.

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Study Citation:	Goldberg, ME; Johnson, HE; Pozzani, UC; Smyth, HF, Jr (1964). Effect of repeated inhalation of vapors of industrial solvents on animal behavior: I. Evaluation of nine solvent vapors on pole-climb performance in rats American Industrial Hygiene Association Journal, 25(4), 369-375
Data Type:	10-day inhalation
HERO ID:	58035

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Outcome assessments were not adequately reported to allow a determination of whether evaluations were performed consistently. The report states that tests made from zero to two hours after exposure gave maximal effects, and results were reported as the quantal response at the time of maximum effect; however, not all time points evaluated were reported.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling were not reported to determine if sampling was adequate for all groups. For example, it's not stated how many of the eight animals per group were evaluated, neither in the text nor in the results table (Table IV).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding status was not reported in this study. Neurobehavioral assessments typically need to be conducted by blinded assessors, however, there was a quantitative aspect to the assessment (i.e., response time). While blinding would have been preferred, it is not as crucial in this case as it is for purely subjective observations.
	Metric 20:	Negative Control Response	Low	× 1	3	Negative control data were not shown for all outcomes; however, negative control data were compared to treatment groups for purposes of determining effects on evaluated outcomes (e.g., body weight, avoidance response, escape response, as shown in Table IV). These uncertainties are unlikely to have a substantial impact on results.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding differences reported among the study groups; however, initial body weight or food/water intake were not reported. Additionally, respiratory rate was not reported, but 1,4-dioxane is a potential respiratory irritant, so I scored this metric as low.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Goldberg, ME; Johnson, HE; Pozzani, UC; Smyth, HF, Jr (1964). Effect of repeated inhalation of vapors of industrial solvents on animal behavior: I. Evaluation of nine solvent vapors on pole-climb performance in rats American Industrial Hygiene Association Journal, 25(4), 369-375
Data Type:	10-day inhalation
HERO ID:	58035

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were reported for body weight data, but not for evaluation of avoidance and escape response data. Mean values with standard deviations were not reported for avoidance and escape response data, so an independent analysis would not be possible.
	Metric 24:	Reporting of Data	Low	× 2	6	Body weight effects were reported (e.g., Table IV) but data were not shown in full. Neurological/behavioral effects, as reported in Table IV, were observed, but data were not reported completely (only %'s affected are shown).
Overall Quality Determination‡			Medium		2.2	
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 7: **Animal toxicity evaluation results of Roy et al 2005 for an in vivo micronucleus assay - main study on genotoxicity outcomes**

Study Citation:	Roy, SK; Thilagar, AK; Eastmond, DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice Mutation Research, 586(1,1), 28-37
Data Type:	In vivo micronucleus assay - main study
HERO ID:	196094

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,4-Dioxane (CAS No. 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	1,4-Dioxane (99.9%, HPLC grade) was obtained from Aldrich Chemical Company (Milwaukee, WI). Batch # not reported, no independent analytical verification.
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%, HPLC grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The negative control group was administered 0.9% NaCl via gavage.
	Metric 5:	Positive Controls	High	× 1	1	Animals in the positive control group were injected intraperitoneally with vinblastine sulfate (0.85 mg/kg per day).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were placed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Storage and preparation were not reported explicitly; based on methods, it appears animals were gavaged with undiluted test substance.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure similar between negative control and exposure groups; gavage volume was not reported. Positive control i.p. injection.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	gavage, once daily for 5 days.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three exposure groups, plus controls. Doses selected based on range-finding study.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Young 21-day-old male CD-1 mice were purchased from Harlan (Indianapolis, Indiana, USA).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Conditions were similar between groups and consistent with standard practices.
	Metric 15:	Number per Group	High	× 1	1	5/group; appropriate for subacute exposure study
Domain 5: Outcome Assessment						

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Study Citation:	Roy, SK; Thilagar, AK; Eastmond, DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice Mutation Research, 586(1,1), 28-37
Data Type:	In vivo micronucleus assay - main study
HERO ID:	196094

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Micronuclei evaluation in bone marrow and hepatic cells; hepatic cells also evaluated for proliferation (BrdU) to evaluation potential origins of micronuclei.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Animals evaluated the same across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were randomized and coded prior to scoring.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported, no deviation from expected reported. Expected results observed in positive controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of initial body weights and food/water intake are unlikely to have an impact on results due to subacute duration and endpoints assessed.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No indication of attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	ANOVA, regression analysis on transformed data; post-hoc Fisher's protected least significant difference. Critical values were determined using a 0.05 probability of type I error.
	Metric 24:	Reporting of Data	High	× 2	2	Data presented quantitatively in tables or figures.
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 8: **Animal toxicity evaluation results of Roy et al 2005 for an in vivo micronucleus assay - range-finding study on genotoxicity, mortality outcomes**

Study Citation:	Roy, SK; Thilagar, AK; Eastmond, DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice Mutation Research, 586(1,1), 28-37
Data Type:	In vivo micronucleus assay - range-finding study
HERO ID:	196094

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,4-Dioxane (CAS No. 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	1,4-Dioxane (99.9%, HPLC grade) was obtained from Aldrich Chemical Company (Milwaukee, WI). Batch # not reported, no independent analytical verification.
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%, HPLC grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Methods states: "an initial range-finding study with three animals per dose was performed at doses ranging from 250 to 5000 mg/kg bw"., but results indicate a control group (0 mg/kg-day). The main study dosed controls with 0.9% NaCl via gavage.
	Metric 5:	Positive Controls	Not Rated	NA	NA	It doesn't appear that the range-finding study used a positive control based on methods and results sections. However, the main study group used a positive control group were injected intraperitoneally with vinblastine sulfate (0.85 mg/kg per day), and saw expected results. Therefore, study design was validated in the lab. For dose-range finding studies, it is likely OK not to have positive control, so I selected N/A.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were placed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Storage and preparation were not reported explicitly; based on methods, it appears animals were gavaged with undiluted test substance.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure similar between negative control and exposure groups; gavage volume was not reported. .
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Undefined number of exposure groups from 250 mg/kg to 5000 mg/kg; mortality observed at >3500 mg/kg. Based on results section, there were 9 dose groups between 250 and 3500 mg/kg, plus a negative control. Unclear if there were any dose groups between 3500 mg/kg and 5000 mg/kg.

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Study Citation: Roy, SK; Thilagar, AK; Eastmond, DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice Mutation Research, 586(1,1), 28-37
Data Type: In vivo micronucleus assay - range-finding study
HERO ID: 196094

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	gavage, assumed once based on reporting and units (mg/kg not mg/kg-day) . Not explicitly stated.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	At least 9 dose groups plus control.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Young 21-day-old male CD-1 mice were purchased from Harlan (Indianapolis, Indiana, USA).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Conditions were similar between groups and consistent with standard practices.
	Metric 15:	Number per Group	High	× 1	1	3/group; adequate for range-finding
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Micronuclei evaluation in bone marrow, mortality
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Animals evaluated the same across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were randomized and coded prior to scoring.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported, no deviation from expected reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of initial body weights and food/water intake are unlikely to have an impact on results due to subacute duration and endpoints assessed.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No indication of attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Unclear if statistics were conducted in range-finding study (main study used ANOVA, regression analysis on transformed data; post-hoc Fisher's protected least significant difference). Data reporting of % micronucleus frequency inadequate for statistical analysis (no SD/SEM data).
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Micronuclei frequency reported as % only (no SD/SEM data); mortality data reported qualitatively only ("some" mortality observed at >3500 mg/kg; unclear if there were doses between 3500 and 5000 mg/kg).

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Study Citation:	Roy, SK; Thilagar, AK; Eastmond, DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice Mutation Research, 586(1,1), 28-37
Data Type:	In vivo micronucleus assay - range-finding study
HERO ID:	196094

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 9: **Animal toxicity evaluation results of Mattie et al 2012 for a 2-week inhalation study on neurological/behavioral, body weight outcomes**

Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	2-wk inhalation study - Neuro and BW
HERO ID:	3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)
	Metric 3:	Test Substance Purity	High	× 1	1	>99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were exposed to clean air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for study type (OECD 412)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were "randomly selected for each exposure group".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Vapor generation method was adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure methods were consistent between groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations reported (Table 4). Exposure chamber concentrations were continuously sampled and the concentration determined approximately every 40 seconds by FTIR analysis for each entire 6 hour exposure.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration consistent with cited guideline (OECD 412)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three exposure groups plus concurrent controls were used (consistent with guideline (OECD 412).. Methods section states that exposure levels were based on levels in the accompanying acute (6-hr) study). However, the discussion states that based on a general lack of findings in acute study, the exposure levels were based on the Kasai et al. (2008) 13-wk study. Doses selected showed dose-response findings, and are considered appropriate.

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Study Citation: Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type: 2-wk inhalation study - Neuro and BW
HERO ID: 3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic, whole-body exposure with 15 complete fresh air changes per hour; individually housed in 690 L chambers. Any aerosols that were formed during vaporization process were captured by a patch of glass wool upstream, so nose-only exposure was not necessary.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Albino inbred Fischer (CDF®) [F344/DuCrl] rats. Age not reported. Based on weights (150-200g for males, 125-175g for females) they were young adults.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.
	Metric 15:	Number per Group	High	× 1	1	16/sex/group; 8/sex sacrificed at end of exposure, 8/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group per sacrifice)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Body weight- at randomization, before each exposure, weekly during recovery, at necropsy Clinical signs of neurotoxicity (autonomic effects, central nervous system effects, and reactivity to handling or sensory stimuli)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessment identical across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling consisted with cited guideline (OECD 412)
	Metric 19:	Blinding of Assessors	Unacceptable	× 1	4	No reporting of blinding status of examiners during subjective assessments of clinical signs of neurotoxicity.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	Body weights and results of clinical signs evaluations were not reported for control or exposure group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Methods section states that evaluations of respiration were conducted, but respiratory rate was not reported (no reporting of clinical signs, or lack thereof). Rated as low since 1,4-dioxane is a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No mortalities were reported. Unlike Acute study, no mention of potential environmental irritants or infection. Because those confounders were reported in the acute study (and not specifically addressed in subacute study), I rated as medium.

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Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	2-wk inhalation study - Neuro and BW
HERO ID:	3563367

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	Unacceptable	× 1	4	No mention of statistical analysis of clinical neurotoxicity evaluation (data not reported). Body weight was reportedly analyzed with Student's t-test and ANOVA (data not reported)
	Metric 24: Reporting of Data	Unacceptable	× 2	8	Body weights and results of clinical signs evaluations were not reported for control or exposure groups.
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 10: **Animal toxicity evaluation results of Mattie et al 2012 for a 2-week inhalation systemic effects study on hepatic, renal, irritation, respiratory, hematological and clinical chemistry outcomes**

Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	2-wk inhalation study - systemic effects
HERO ID:	3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)
	Metric 2:	Test Substance Source	Medium	× 1	2	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)
	Metric 3:	Test Substance Purity	High	× 1	1	>99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were exposed to clean air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for study type (OECD 412)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were "randomly selected for each exposure group".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Vapor generation method was adequately reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure methods were consistent between groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations reported (Table 4). Exposure chamber concentrations were continuously sampled and the concentration determined approximately every 40 seconds by FTIR analysis for each entire 6 hour exposure.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration consistent with cited guideline (OECD 412)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three exposure groups plus concurrent controls were used (consistent with guideline (OECD 412).. Methods section states that exposure levels were based on levels in the accompanying acute (6-hr) study). However, the discussion states that based on a general lack of findings in acute study, the exposure levels were based on the Kasai et al. (2008) 13-wk study. Doses selected showed dose-response findings, and are considered appropriate.

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Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	2-wk inhalation study - systemic effects
HERO ID:	3563367

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic, whole-body exposure with 15 complete fresh air changes per hour; individually housed in 690 L chambers. Any aerosols that were formed during vaporization process were captured by a patch of glass wool upstream, so nose-only exposure was not necessary.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Albino inbred Fischer (CDF®) [F344/DuCrl] rats. Age not reported. Based on weights (150-200g for males, 125-175g for females) they were young adults.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.
	Metric 15:	Number per Group	High	× 1	1	16/sex/group; 8/sex sacrificed at end of exposure, 8/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group per sacrifice)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Hepatic, Renal - Clinical chemistry, OW, HP Respiratory - HP of entire respiratory tract, including nasal sections (Cited guideline indicates that BALF should be done; however, study authors did not indicate that this was done. The extensive histopathological evaluation is considered adequate to assess this endpoint) Hematology - at sacrifice
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessment identical across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling consisted with cited guideline (OECD 412)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only non-subjective outcomes and initial histopathological evaluations performed; blinding not necessary.
	Metric 20:	Negative Control Response	High	× 1	1	Quantitative lesion data reported. Qualitative statement regarding no statistically significant changes in clinical chemistry or hematology covers both control and exposure groups. Organ weight data not reported for any group (downgraded in data presentation metric, not here)
Domain 6: Confounding / Variable Control						

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Study Citation:	Mattie, DR; Bucher, TW; Carter, AL; Stoffregen, DE; Reboulet, JE (2012). Acute inhalation toxicity study of 1, 4-dioxane in rats (Rattus norvegicus) GRA and I(20), 29
Data Type:	2-wk inhalation study - systemic effects
HERO ID:	3563367

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Methods section states that evaluations of respiration were conducted, but respiratory rate was not reported (no reporting of clinical signs, or lack thereof). Rated as low since 1,4-dioxane is a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No mortalities were reported. Unlike Acute study, no mention of potential environmental irritants or infection. Because those confounders were reported in the acute study (and not specifically addressed in subacute study), I rated as medium.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Lesion incidence compared with Fisher's exact test. Continuous data analyzed by t-test and ANOVA.
	Metric 24:	Reporting of Data	Medium	× 2	4	Quantitative reporting of lesions. Qualitative negative result reporting for hematology and clinical chemistry. Incidence data reported, but individual animal histopathology results not reported. Organ weights not reported. Likely no effect (no impact on outcome), so rated as medium.
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 11: **Animal toxicity evaluation results of Dow et al 1989 for a repeat dose in vivo DNA synthesis study on hepatic, genotoxicity, body weight outcomes**

Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Repeat dose in vivo DNA synthesis
HERO ID:	4158030

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Reported only as "1,4-dioxane".
	Metric 2:	Test Substance Source	Medium	× 1	2	Baker Chemical Company; no batch number, but purity was analyzed by study laboratory
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle (saline) control was used
	Metric 5:	Positive Controls	High	× 1	1	Known genotoxic agent dimethylnitrosamine (DMN) was used as a positive control
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were computer randomized into treatment groups in all experiments
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Storage details not reported. Mixed with drinking water. No details on frequency of drinking water preparation.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions consistent between groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Study authors report drinking water provided an average dose of 0, 10, or 1000 mg/kg-d. Nominal doses in drinking water were not reported. Data used to calculate average daily dose was not provided.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	11 weeks, 7d/wk
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 doses plus negative and positive control
	Metric 12:	Exposure Route and Method	Medium	× 1	2	No rationale was provided for switching from gavage (accompanying acute study) to repeat-dose drinking water study. Other compounds (HCBD, DMN) were administered via gavage for both studies. However, BWG was decreased by ~45-55% following single gavage administration of 1000 mg/kg; this BW effect was not observed with drinking water administration of 1000 mg/kg over 11 weeks. SO perhaps the change in route was due to the decreased body weight associated with gavage administration.
Domain 4: Test Organism						

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Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Repeat dose in vivo DNA synthesis
HERO ID:	4158030

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	High	× 2	2	Male SD rats (Spartan Research). Based on weight (180-260g), they were adult animals.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry was consistent between groups (wire cages, environmentally controlled rooms, food and water ad libitum). Number of rats/cage was not reported, environmental conditions not reported.
	Metric 15:	Number per Group	High	× 1	1	5-6/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Genotox, organ weight, and histology of liver (cancer target organ); body weight and food consumption also monitored.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	5-6/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only non-subjective and initial histological evaluations; blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	negative control response was reported; no deviations from normal were reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Initial BW 180-260g (not reported per group). Body weight gains similar between groups during study.
	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	Continuous data were compared by Dunnett's t-test. No statistical analysis of histopathological findings. Histological findings only reported qualitatively.
	Metric 24:	Reporting of Data	High	× 2	2	DNA synthesis, liver weight, and BWG reported quantitatively with statistics. Histopathological findings reported qualitatively (present or absent at dose).
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.5~~	
Extracted			Yes			

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Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Repeat dose in vivo DNA synthesis
HERO ID:	4158030

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Downgraded based on the uncertainty in the actual doses (see metric 9)."

Table 12: **Animal toxicity evaluation results of Itoh 2019 - in vivo genotoxicity assay - micronucleus test**

Study Citation:	Itoh, S; Hattori, C (2019). In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 837 8-14
Data Type:	In vivo genotox assays
HERO ID:	5072318

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,4-dioxane (CAS No. 123-91-1)
	Metric 2:	Test Substance Source	High	× 1	1	Wako Pure Chemical Industries, Ltd. (Osaka, Japan)
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control
	Metric 5:	Positive Controls	High	× 1	1	For liver micronucleus: diethylnitrosamine [DNN] (juvenile and partial hepatectomy methods), carbendazim (partial hepatectomy method) Bone marrow micronucleus: cyclophosphamide monohydrate [CP] Pig-a assay: 7,12-dimethylbenz[a]anthracene [DMBA]
	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 test chemical and DEN were dissolved in water for injection. Carbendazin was suspended on 0.5% methylcellulose. CP and DMBA were dissolved and suspended in saline.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across exposure groups for each experiment.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	0, 1000, 2000, or 3000 mg/kg
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Liver MN juvenile method: dosed on Day 1 and Day 2, hepatocyte isolation on Day 6 Liver-MN PH method: Exposed once either the day before PH or day after PH; hepatocyte isolation 5 days after PH Bone marrow MN: Exposed once (Day 1) with bone marrow removed Day 2 or 3 Pig-a test: Exposed once (Day 1) with peripheral blood obtained on Days -1, 15, and 30
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	0, 1000, 2000, or 3000 mg/kg based on previous reports
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage at dose volume of 10 mL/kg

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Study Citation:	Itoh, S; Hattori, C (2019). In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 837 8-14
Data Type:	In vivo genotox assays
HERO ID:	5072318

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Male F344/DuCrlCrlj rats, 4- to 8-wks of age; Charles River Laboratories Japan, Inc.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	This study was conducted in compliance with the following law and guidelines; "Law Concerning the Protection and Control of Animals", Japanese Law No. 105, October 1, 1973, revised on June 22, 2005
	Metric 15:	Number per Group	High	× 1	1	4-5/group per test
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	High for genotoxicity: evaluated with 4 tests - two liver MN assays, a bone marrow MN assay, and blood Pig-a mutation assay Unacceptable for liver toxicity (only relative liver weight evaluated)
	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	All quantitative measures
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW not reported; not likely to have substantial impact
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	MN: two-tailed Fisher's exact test and two-tailed Cochran-Armitage trend test % IE: Wilcoxon's rank sum Pig-a: Bartlett's test to evaluate the homogeneity of variance; analyzed by a parametric Dunnett's test when the variance was homogeneous or by a Steel's (nonparametric Dunnett's) test when it was not
	Metric 24:	Reporting of Data	High	× 2	2	Graphical reporting of all genotox data; quantitative reporting for relative liver weight data
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	Itoh, S; Hattori, C (2019). In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 837 8-14
Data Type:	In vivo genotox assays
HERO ID:	5072318

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

3 Subchronic (30-90 days)

Table 13: **Animal toxicity evaluation results of Kasai et al 2008 for a 13-week inhalation study on hepatic, renal, hematology, clinical chemistry, respiratory, body weight, mortality outcomes**

Study Citation:	Kasai, T; Saito, M; Senoh, H; Umeda, Y; Aiso, S; Ohbayashi, H; Nishizawa, T; Nagano, K; Fukushima, S (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats Inhalation Toxicology, 20(10), 961-971
Data Type:	13-week inhalation
HERO ID:	195044

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reagent grade 1,4-Dioxane (>99% pure); liquid
	Metric 2:	Test Substance Source	High	× 1	1	Obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Batch number not provided, but identity and composition verified by laboratory using GC-MS.
	Metric 3:	Test Substance Purity	High	× 1	1	Reagent grade 1,4-Dioxane (>99% pure); analyzed for purity and stability using GC-MS before and after use. Butylhydoxytoluene was detected in 1,4-dioxane liquid by GC-MS (1.3 ppm w/w), but it was not detected in air samples collected from inhalation air samples.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent control group exposed to clean air under same conditions as test groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control group is not needed in standard 13-wk inhalation study (see OECD guideline 413)
	Metric 6:	Randomized Allocation	High	× 1	1	stratified randomization into 8 body-weight-matched groups, each comprised of 10 rats/sex
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Detailed description of vapor generation; chamber concentrations of 1,4-dioxane monitored every 15 minutes during exposure;
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions identical between groups (except exposure levels). All animals in an exposure group were exposed simultaneously (exposure chamber held 20 individual cages).

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Study Citation:	Kasai, T; Saito, M; Senoh, H; Umeda, Y; Aiso, S; Ohbayashi, H; Nishizawa, T; Nagano, K; Fukushima, S (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats Inhalation Toxicology, 20(10), 961-971
Data Type:	13-week inhalation
HERO ID:	195044

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytical concentrations reported, and within 1% of target. Chamber concentrations of 1,4-dioxane monitored every 15 minutes during exposure. Accuracy and precision of the actual concentrations of 1,4-dioxane in the exposure chamber were kept by periodic injection of the certified standard 1,4-dioxane gas (Takachiho Co., Ltd., Tokyo) into the gas chromatograph for the calibration curve of 1,4-dioxane.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Consisted with cited OECD guideline 413 (6 h/d, 5 d/wk, 13 wk)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Adequate number of exposure groups (n=7 plus control). However, lowest dose was identified as a LOAEL (no NOAEL identified), and the highest dose was 100% lethal (high dose too high). However, the number of dose groups provides dose response data (increased effects/incidence with increasing dose).
	Metric 12:	Exposure Route and Method	High	× 1	1	Detailed description of vapor generation and whole-body exposure conditions (1060 L exposure chambers, housed 20 individual cages).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Six-week-old F344/DuCrj rats of both sexes (obtained at 4-weeks of age)
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing conditions described adequately; same conditions in control and exposure groups.
	Metric 15:	Number per Group	High	× 1	1	10/sex/group, as per cited OECD guideline 413
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	PECO endpoints: Renal - clinical chemistry, urinalysis, organ weight, histology Hepatic - clinical chemistry, urinalysis, organ weight, histology Neuro - clinical signs, brain, spinal cord, and nerve histo, assumed brain weight due to cited OECD 413 guideline Other endpoints: Respiratory - lung weight, histo of entire respiratory tract (including nasal sections) Hemato, BW, mortality - adequately evaluated

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Study Citation:	Kasai, T; Saito, M; Senoh, H; Umeda, Y; Aiso, S; Ohbayashi, H; Nishizawa, T; Nagano, K; Fukushima, S (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats Inhalation Toxicology, 20(10), 961-971
Data Type:	13-week inhalation
HERO ID:	195044

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups as described in methods section with exception of high-dose group due to 100% lethality by week 1 (histology was performed at death). There were no mortalities in other groups. Due to 6 exposure groups other than the high-dose group, loss of this high dose group to 13 week assessments does not alter evaluation or interpretation of the results.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling consistent with cited OECD guideline 413.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding status of assessors was not reported, Evaluated endpoints included non-subjective metrics and initial histopathology review, so blinding was not needed.
	Metric 20:	Negative Control Response	High	× 1	1	Control results were reported, and within expected biological variation.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial groups were weight-matched. No abnormal clinical signs were reported in surviving groups (all high-dose animals died within a week), so altered breathing with exposure is unlikely. However, respiratory rate (or lack of bradypnea) was not specifically mentioned so I downgraded to medium.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Mortality was limited to the high-exposure group, and was attributed to exposure-related effects (renal failure)
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Continuous variables were evaluated using Dunnett's test and dichotomous variables were evaluated using chi-square. 2-sided analysis with p-values of 0.05 and 0.01 was performed.

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Study Citation:	Kasai, T; Saito, M; Senoh, H; Umeda, Y; Aiso, S; Ohbayashi, H; Nishizawa, T; Nagano, K; Fukushima, S (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats Inhalation Toxicology, 20(10), 961-971
Data Type:	13-week inhalation
HERO ID:	195044

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	Only some of the blood parameters (clinical chemistry, hematology) were reported quantitatively. It is assumed that other parameters listed in OECD 413 were evaluated and no exposure-related effects were found, but results were not reported. A slight decrease in urinary protein was qualitatively reported; no other urinalysis results were reported (again, assumed that endpoints in OECD 413 were evaluated). Relative organ weights and histology were reported quantitatively (for exposure-related effects). Male kidney and male and female nervous system histology were not reported, but it is implied that no exposure-related effects were observed other than respiratory tract and liver in males and females and kidneys in females (see histopathology section in results).
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 14: **Animal toxicity evaluation results of Kano et al 2008 for a 13-week oral toxicity of 1,4-d in rats and mice study**

Study Citation:	Kano, H; Umeda, Y; Saito, M; Senoh, H; Ohbayashi, H; Aiso, S; Yamazaki, K; Nagano, K; Fukushima, S (2008). Thirteen-week oral toxicity of 1,4-dioxane in rats and mice Journal of Toxicological Sciences, 33(2), 141-153
Data Type:	13-week oral toxicity of 1,4-D in rats and mice
HERO ID:	196245

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name; no concern with different forms or mixtures.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from commercial source. and its purity established by IS and GC.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance obtained from commercial source; purity >99.0% verified by IS and GC.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Control group received vehicle (deionized water); all groups were body-weight matched (stratified randomization).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not indicated for study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Group assignments by stratified randomization into body-weight matched groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test material was analyzed for stability before and after use; no decomposition products or impurities identified. Test material prepared twice per week. Analysis of test material immediately after preparation showed concentrations 94.6-102.9% of target; analysis of test material 4 days after preparation showed concentrations 92.8-101.1% of initial concentrations.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Daily water intake calculated as difference between weight of water remaining in bottle 3-4 days after preparation divided by number of days.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Intake of 1,4-D was estimated by study authors based on nominal concentration, body weight (measured once weekly), and water intake (measured every 3-4 days).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency was not specified but is inferred to be 7 days per week; duration specified as 13 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The rationale for dose selection was not stated, but the study included 5 non-zero exposure concentrations across a 39-fold range. Exposure levels included those high enough to induce effects and low enough to identify a NOAEL.

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Study Citation: Kano, H; Umeda, Y; Saito, M; Senoh, H; Ohbayashi, H; Aiso, S; Yamazaki, K; Nagano, K; Fukushima, S (2008). Thirteen-week oral toxicity of 1,4-dioxane in rats and mice Journal of Toxicological Sciences, 33(2), 141-153
Data Type: 13-week oral toxicity of 1,4-D in rats and mice
HERO ID: 196245

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was reported and appropriate (drinking water).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal species, strain, age, and source were all reported and appropriate for subchronic toxicity evaluation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	No differences between groups in animal husbandry conditions were reported. Animals were housed individually.
	Metric 15:	Number per Group	High	× 1	1	Study used 10 animals/sex/group, which exceeds numbers recommended by OECD (5/sex/grp)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was described in detail including organs/endpoints, methods, instrumentation, stains, and timing. Endpoints evaluated were sensitive for systemic toxicity.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in protocol execution were noted in the report.
	Metric 18:	Sampling Adequacy	High	× 1	1	All standard endpoints were evaluated in all animals of all exposure groups. ALtered hepatic foci evaluated in subsets of high exposure and control groups.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	There were no subjective outcomes evaluated.
	Metric 20:	Negative Control Response	High	× 1	1	Adequately reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Unacceptable	× 2	8	In both male and female rats and mice, drinking water intakes in the top two exposure groups were at least 20% lower than control intakes.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Animal attrition was limited to two deaths (one rat and one mouse). No infections or other health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the data.
	Metric 24:	Reporting of Data	High	× 2	2	Data for all groups on exposure-related findings were reported. Measures of variation and numbers of animals examined were reported.
Overall Quality Determination‡			~~Unacceptable~~⋆⋆ ⟶ Medium§		~~1.2~~	

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Study Citation:	Kano, H; Umeda, Y; Saito, M; Senoh, H; Ohbayashi, H; Aiso, S; Yamazaki, K; Nagano, K; Fukushima, S (2008). Thirteen-week oral toxicity of 1,4-dioxane in rats and mice Journal of Toxicological Sciences, 33(2), 141-153
Data Type:	13-week oral toxicity of 1,4-D in rats and mice
HERO ID:	196245

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

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

§ Evaluator's explanation for rating change: "Although there was a dose-related decrease in water intake that exceeded 20

4 Chronic (>90 days)

Table 15: **Animal toxicity evaluation results of Argus et al 1965 for a cancer bioassay-liver, kidney, blood study on cancer outcomes**

Study Citation:	Argus, MF; Arcos, JC; Hoch-Ligeti, C (1965). Studies on the carcinogenic activity of protein-denaturing agents: Hepatocarcinogenicity of dioxane Journal of the National Cancer Institute, 35(6), 949-958
Data Type:	Cancer bioassay-liver, kidney, blood
HERO ID:	17009

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and chemical formula and structure
	Metric 2:	Test Substance Source	Medium	× 1	2	Eastman organic chemical number was reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Details regarding the negative control group were not reported, based on the study design, it is not clear that the animals were treated in any manner making direct comparison among results challenging.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The metric is not applicable.
	Metric 6:	Randomized Allocation	Low	× 1	3	How animals were allocated was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Limited preparation (1% in drinking water) information was reported and storage information was not provided. Given that 1,4-dioxane is stable in water, the incomplete information is not expected to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Treated animals had access to drinking water continuously
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The maximum dose/rat, approximate daily water intake rate, and body weight range at the end of the study were reported, so approximation of dose could be calculated.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Data found in Table 1.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Not Rated	NA	NA	Only one treatment dose was used
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure through drinking water was acceptable as 1,2-dioxane can leach into and remain in water
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Animal source, species, strain, sex, life-stage, and body weight range were reported. Specific age and health status was not reported.

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Study Citation:	Argus, MF; Arcos, JC; Hoch-Ligeti, C (1965). Studies on the carcinogenic activity of protein-denaturing agents: Hepatocarcinogenicity of dioxane Journal of the National Cancer Institute, 35(6), 949-958
Data Type:	Cancer bioassay-liver, kidney, blood
HERO ID:	17009

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Limited husbandry conditions were reported, but appear to be similar among the groups.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number was lower than the typical number (26 vs 30 for cancer bioassay). It is unclear if this is the initial number of animals/group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Limited details regarding the complete necropsy and histological investigation were reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Based on the study report, it is inferred that outcome assessment was consistent.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	Biological responses were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reported of initial body weight and specific water intake is not 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 were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not conducted, but some data were provided which could be used to do an independent analysis (incidence of rats with tumors)
	Metric 24:	Reporting of Data	Medium	× 2	4	Tabular data for tumor outcomes was reported, all other data were described in the text and incidence and severity data were not reported.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 16: **Animal toxicity evaluation results of Kociba et al 1974 for a 2-year drinking water study study on cancer, hepatic, renal, hematological and immune, body weight, mortality outcomes**

Study Citation:	Kociba, RJ; Mccollister, SB; Park, C; Torkelson, TR; Gehring, PJ (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286
Data Type:	2-year drinking water study
HERO ID:	62929

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Clearly identifies substance as 1,4-dioxane
	Metric 2:	Test Substance Source	Medium	× 1	2	Compound obtained from The Dow Chemical Co. (batch no. not reported).
	Metric 3:	Test Substance Purity	High	× 1	1	Purity not reported, but stock samples were analyzed for impurities at 6 different times during 2-year study. The following impurities were reported in stock solutions: hydrogen peroxide (10-340 ppm), crotonaldehyde (220-1340 ppm), 2-methyl-1,3-dioxolane (6-108 ppm), water (10-90 ppm). No acetaldehyde was detected. So purity was >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated controls were given regular drinking water.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not warranted by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Storage conditions prior to opening were provided. Samples were used within 1 week after bottles were opened. Drinking water solutions were prepared twice weekly during the first year and weekly during the second year.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Drinking water was available ad libitum to all exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Daily water consumption was recorded, with rates calculated for 3 different time periods of the 2-year study (Days 1-113, 114-198, 446-460). These values plus BW data were used to calculate daily doses of 1,4-dioxane in mg/kg/day. Drinking water samples were analyzed for 1,4-dioxane content "periodically" via gas liquid chromatography.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	2 yr study; drinking water available ad libitum

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Study Citation: Kociba, RJ; Mccollister, SB; Park, C; Torkelson, TR; Gehring, PJ (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286
Data Type: 2-year drinking water study
HERO ID: 62929

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 dose groups - low dose did not induce toxic effects or tumors; mid-dose induced some toxic effects, high-dose induced tumors.
	Metric 12:	Exposure Route and Method	High	× 1	1	drinking water administration
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	6-8 wk old Sherman rats; male and female
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Information on husbandry limited to "maintained in animal care facilities fully accredited by the American Association for Accreditation of laboratory Animal Care". All rats were maintained under these "approved conditions". Water and standard feed available ad libitum.
	Metric 15:	Number per Group	High	× 1	1	60/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Cancer: complete histological analysis, sufficient duration of study Renal: OW, histopathology Hepatic: OW, histopathology Hematology, Bd wt, mortality - adequately assessed
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The same protocols were used for control and exposure groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Adequate numbers were used in all groups. Effective number of animals for tumor analysis was calculated.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	All evaluations were non-subjective or initial histopathological evaluations.
	Metric 20:	Negative Control Response	High	× 1	1	Control results reported, no noted deviations from expectation.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Based on graphically reported data, BW were similar between groups at study initiation. Decreased water consumption was observed in high-dose group (10-12% during Days 1-198) and mid-dose group females (8% from days 114-198).
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Decreased survival during the first 4 months of the study in the high-dose group attributed to exposure (hepatic and renal toxicity); mortality was comparable to control in low- and mid-dose group.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Kociba, RJ; Mccollister, SB; Park, C; Torkelson, TR; Gehring, PJ (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286
Data Type:	2-year drinking water study
HERO ID:	62929

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	High	× 1	1	Tumors evaluated using Fisher's Exact probability test. Survival rates were compared using Chi-Square and Fisher's Exact probability test. Student t test was used to compared continuous variables.
	Metric 24:	Reporting of Data	Medium	× 2	4	Cancer - tumor incidence data reported adequately Hepatic - significant change in liver weight reported qualitatively only, nonneoplastic changes reported qualitatively only Renal - no change in OW (qualitative), nonneoplastic changes reported qualitatively only Hematological - no change in parameters (qualitative) Bd wt and Mortality reported graphically
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 17: **Animal toxicity evaluation results of NCI et al 1978 for a cancer bioassay- male rats study on cancer outcomes**

Study Citation:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity					
Data Type:	Cancer bioassay- male rats					
HERO ID:	62935					
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 reported, including lot numbers. The test substance (one of two lots) was analyzed to confirm identity and purity (using vapor phase chromatography and spectrometry).
	Metric 3:	Test Substance Purity	High	× 1	1	The purity (one of two lots) was 99.9%. The test substance was tested for specific impurities (sodium diethylthiocarbamate, and peroxide); these impurities were generally present at 0.001% or less. However one lot showed peroxide levels of 0.1% after study completion. This deficiency is not likely to substantially impact the study results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	Matched drinking water control groups were used. However, groups were not placed on study at the same time. Control and high-dose male rats were placed on study later than other groups (by 1 year). Based on data presented graphically in the study report, the weights of low-dose male rats differed from the body weights of control and high-dose animals at study week 0.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control group not indicated by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animals were assigned to control or dose groups "according to a series of random numbers;" there were deficiencies regarding the allocation method that may impact the study results (e.g. allocation by animal number).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation and storage conditions were not reported in exhaustive detail ("dioxane solutions prepared in tap water twice per week and stored in polyethylene containers"). Test substance stability was demonstrated via analyses conducted several months after study completion; however, data on stability of the test substance under the conditions of administration (in water) were not provided.

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- male rats
HERO ID: 62935

Domain		Metric	Rating†	MWF*	Score	Comments††
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Dosed water or tap water was available ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	As per applicable guideline, water consumption should be measured at least weekly for the first 13 weeks and at least monthly thereafter. Although doses in mg/kg-day were provided, these doses were based on water consumption determined at intervals during the second year of the bioassay only (and using 20% of the animals as a representative sample). The study report indicates that "there were wide fluctuations in intake at different time periods within groups."
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	As per applicable guideline, the duration of the study will normally be 24 months for rats . In this study, rats were dosed for 110 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Concentrations were chosen based on the results of previous studies (by Argus et al. 1965). As per applicable guideline, at least three dose levels and a concurrent control should be used; however, the PECO statement requires at least 2 dose groups and a control. The study used two dose groups and a control; however, the control groups was not concurrent (i.e.. data for only 1 quantitative dose group and controls in male rats were concurrent). The difference between the low- and high-dose in rats was also not two-fold (as intended). These factors are likely to have an impact on the study results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route of exposure was reported (i.e. drinking water); however, no rationale was provided. The applicable guideline considers drinking water to be a valid route of administration.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, health status, sex, age, and body weights at study week 0 (provided graphically) were reported. Animals were obtained from a commercial laboratory. These animals were appropriate models for the evaluation of carcinogenicity (although not the same rat strain used in previous studies).

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- male rats
HERO ID: 62935

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions (temperature, humidity, light cycles) were reported, and appear to be adequate (compared to guideline recommendations;) and the same for control and dosed groups. The applicable guideline indicates that animals should be housed individually or in small groups. The study report indicates that rats were housed 4 per cage. This is unlikely to have had a substantial impact on results (there were no indications of injuries or death due to overcrowding).
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was lower than the typical number used in carcinogenicity studies in rats (35/sex/group compared to 50/sex/group recommended by guideline). However, the study report indicated that animal numbers were adequate for statistical analyses (related to carcinogenicity).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Animals from all dose groups were subjected to gross and microscopic pathology evaluations. The number of tissues evaluated was not as comprehensive as that recommended by guideline (at least in low-dose rats), but this deficiency is not likely to substantially impact the study results.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Surviving rats were sacrificed at 110-117 weeks. The tissues from some animals were not evaluated (particularly in animals that died early). Therefore, the numbers of animals subjected to histopathological evaluations (with respect to specific organs or tissues) are not the same as the number of animals placed on study.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Histopathological examinations were performed on dosed groups and controls. Although details were not reported (e.g. the numbers of slides evaluated, individual animal data available but not provided). these deficiencies are not likely to substantially impact the study results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not reported, but is not required for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate (showing no or low incidences of lesions).
Domain 6: Confounding / Variable Control						

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- male rats
HERO ID: 62935

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Doses administered to low- and high-dose groups of rats were not reflective of the intended doses owing (at least in part) to decreased palatability (water consumption data were not provided). Initial body weights were not explicitly reported (weights at study week 0 were shown graphically). Rats were housed in the same room with rats administered dibenzodioxin, 2,7-dichlorobenzodioxin, and 1,2,3,4,6,7,8,9-octachlorodibenzodioxin.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	The study report indicated that dosed animals showed pneumonia more frequently than controls. The study authors suggested that the development of pneumonia in rats may have been related to the prevalence of nasal carcinomas.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Procedures used for statistical analyses were described in detail. and appear to be relevant for some endpoints (i.e. cancer; the focus of this study). Owing to differences in the timing of dosing, carcinogenicity data for high-dose male rats were compared to controls only (and not to low-dose males). Statistical analyses for some endpoints (e.g. mortality) appear to consider all groups of male rats, even though dosing was not concurrent. Incidences of non-neoplastic lesions were not subjected to statistical analyses.
	Metric 24:	Reporting of Data	High	× 2	2	Data for relevant outcomes (carcinogenicity data) were provided by exposure group and sex. Data for other endpoints (e.g. mortality, water consumption) were not adequately reported.
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 18: **Animal toxicity evaluation results of NCI et al 1978 for a cancer bioassay- female rats and male and female mice study on cancer outcomes**

Study Citation:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity					
Data Type:	Cancer bioassay- female rats and male and female mice					
HERO ID:	62935					
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 reported, including lot numbers. The test substance (one of two lots) was analyzed to confirm identity and purity (using vapor phase chromatography and spectrometry).
	Metric 3:	Test Substance Purity	High	× 1	1	The purity (one of two lots) was 99.9%. The test substance was tested for specific impurities (sodium diethylthiocarbamate, and peroxide); these impurities were generally present at 0.001% or less. However one lot showed peroxide levels of 0.1% after study completion. This deficiency is not likely to substantially impact the study results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Matched drinking water control groups were used. However, groups were not placed on study at the same time. Control female rats were placed on study later than other groups (by 5 weeks); it was noted that groups of mice were placed on study "not more than 7 weeks apart"). Based on data presented graphically in the study report, the weights of low-dose mice differed from the body weights of control and high-dose animals at study week 0.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control group not indicated by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animals were assigned to control or dose groups "according to a series of random numbers;" there were deficiencies regarding the allocation method that may impact the study results (e.g. allocation by animal number).
Domain 3: Exposure Characterization						

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- female rats and male and female mice
HERO ID: 62935

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation and storage conditions were not reported in exhaustive detail ("dioxane solutions prepared in tap water twice per week and stored in polyethylene containers"). Test substance stability was demonstrated via analyses conducted several months after study completion; however, data on stability of the test substance under the conditions of administration (in water) were not provided.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Dosed water or tap water was available ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	As per applicable guideline, water consumption should be measured at least weekly for the first 13 weeks and at least monthly thereafter. Although doses in mg/kg-day were provided, these doses were based on water consumption determined at intervals during the second year of the bioassay only (and using 20% of the animals as a representative sample). The study report indicates that "there were wide fluctuations in intake at different time periods within groups."
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	As per applicable guideline, the duration of the study will normally be 24 and 18 months for rats and mice, respectively. In this study, rats were dosed for 110 weeks and mice were dosed for 90 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Concentrations were chosen based on the results of previous studies (by Argus et al. 1965). However, as per applicable guideline, at least three dose level;s and a concurrent control should be used (the PECO statement requires at least 2 dose groups and a control). The study used two dose groups and a control. The study report noted that the average daily intake of the test substance in high-dose male mice was only slightly higher than that of low-dose mice (estimated 830 vs. 720 mg/kg-day). The difference between the low- and high-dose in rats was also not two-fold (as intended). These factors are likely to have an impact on the study results.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was reported (i.e. drinking water); however, no rationale was provided. The applicable guideline considers drinking water to be a valid route of administration.
Domain 4: Test Organism						

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- female rats and male and female mice
HERO ID: 62935

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, health status, sex, age, and body weights at study week 0 (provided graphically) were reported. Animals were obtained from a commercial laboratory. These animals were appropriate models for the evaluation of carcinogenicity (although not the same rat strain used in previous studies).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions (temperature, humidity, light cycles) were reported, and appear to be adequate (compared to guideline recommendations;) and the same for control and dosed groups. The applicable guideline indicates that animals should be housed individually or in small groups. The study report indicates that rats were housed 4 per cage and mice 10 per cage. This is unlikely to have had a substantial impact on results (there were no indications of injuries or death due to overcrowding).
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was lower than the typical number used in carcinogenicity studies in rats (35/sex/group compared to 50/sex/group recommended by guideline). However, the study report indicated that animal numbers were adequate for statistical analyses (related to carcinogenicity).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Animals from all dose groups were subjected to gross and microscopic pathology evaluations. The number of tissues evaluated was not as comprehensive as that recommended by guideline, but this deficiency is not likely to substantially impact the study results.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Surviving rats and mice were sacrificed at 110-117 and 90-93 weeks, respectively. The tissues from some animals were not evaluated (particularly in animals that died early). Therefore, the numbers of animals subjected to histopathological evaluations (with respect to specific organs or tissues) are not the same as the number of animals placed on study.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Histopathological examinations were performed on dosed groups and controls. Although details were not reported (e.g. the numbers of slides evaluated, individual animal data available but not provided). these deficiencies are not likely to substantially impact the study results.

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Study Citation: NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type: Cancer bioassay- female rats and male and female mice
HERO ID: 62935

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not reported, but is not required for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate (showing no or low incidences of lesions).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Doses administered to low- and high-dose groups of rats and mice were not reflective of the intended doses owing (at least in part) to decreased palatability (water consumption data were not provided). Initial body weights were not explicitly reported (weights at study week 0 were shown graphically). Rats and mice were housed in the same room with rats administered dibenzodioxin, 2,7-dichlorobenzodioxin, and 1,2,3,4,6,7,8,9-octachlorodibenzodioxin.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	The study report indicated that dosed animals showed pneumonia more frequently than controls. The study authors suggested that the development of pneumonia in rats may have been related to the prevalence of nasal carcinomas.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Procedures used for statistical analyses were described in detail. and appear to be relevant for some endpoints (i.e. cancer; the focus of this study). Statistical analyses for some endpoints (e.g. mortality) appear to consider all groups of rats and mice, even when dosing was not necessarily concurrent. Incidences of non-neoplastic lesions were not subjected to statistical analyses.
	Metric 24:	Reporting of Data	High	× 2	2	Data for relevant outcomes (carcinogenicity data) were provided by exposure group and sex. Data for other endpoints (e.g. mortality, water consumption) were not adequately reported.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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Study Citation:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity
Data Type:	Cancer bioassay- female rats and male and female mice
HERO ID:	62935

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 19: **Animal toxicity evaluation results of Torkelson et al 1974 for a chronic toxicity/carcinogenicity assay in rats study on mortality, body weight, hematological and immune, clinical chemistry/biochemical, cancer outcomes**

Study Citation:	Torkelson, TR; Leong, BKJ; Kociba, RJ; Richter, WA; Gehring, PJ (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298
Data Type:	Chronic toxicity/carcinogenicity assay in rats
HERO ID:	94807

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported. Details regarding analytical verification of test substance identity were not provided, but are not likely to impact the study results.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reportedly 99.9%; therefore, any effects observed are likely due to the nominal test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group (rats exposed to filtered air only).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not indicated by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not indicate how animals were allocated to study groups,
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Samples of the test substance were padded with nitrogen and stored in bottles until opened for use; once opened the test substance was used within one week. The methods and general types of equipment used to generate the test substance as a vapor were reported (without detail); this is not likely to impact the study results.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were generally reported (same exposure frequency, consistent chamber design). There were 4 animals per cage during and in between exposures; time of day of exposures occurred was not specified.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytical, nominal, and target concentrations were reported. The actual concentration did not deviate widely (within 10%). The target concentration was 0.36 mg/L; the actual concentration was 0.4 mg/L (obtained from repeated infared spectrometric analyses).

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Study Citation: Torkelson, TR; Leong, BKJ; Kociba, RJ; Richter, WA; Gehring, PJ (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298
Data Type: Chronic toxicity/carcinogenicity assay in rats
HERO ID: 94807

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were suited to the study type and outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	The dose groups and spacing are not relevant for assessment. As per applicable guideline, there should be 3 dose groups and a control; the PECO statement specifies the need for two dose groups and a control. This study used one group exposed to the test substance and a control group. The number of exposure groups is not adequate to evaluate exposure-response relationships. The concentration of the test substance used in the study was based on the threshold limit value (ACGIH), but was not high enough to elicit toxicity.
	Metric 12:	Exposure Route and Method	High	× 1	1	Rats were exposed to the test substance under dynamic exposure conditions.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	General information regarding test animal characteristics (age, health status) were not reported, but are unlikely to impact the study results. The test animal species, strain, and sex were reported. Mean body weights at month 0 of the experiment are shown graphically in the study report.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported in sufficient detail to determine if conditions were the same/adequate between control and exposed groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per groups was reported and adequate for the study type. Typically 50/sex/group are used for rodent cancer bioassays; this study used 288 rats/sex/exposure group and 192 rats/sex/group for controls.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes appear to have been assessed consistently across groups (same time after initial exposure) and using the same protocols.
	Metric 18:	Sampling Adequacy	High	× 1	1	Endpoints (including hematology and clinical chemistry, gross and microscopic pathology) were evaluated in all surviving animals.

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Study Citation:	Torkelson, TR; Leong, BKJ; Kociba, RJ; Richter, WA; Gehring, PJ (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298
Data Type:	Chronic toxicity/carcinogenicity assay in rats
HERO ID:	94807

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for initial histopathology examinations (other endpoints evaluated were not subjective).
	Metric 20:	Negative Control Response	High	× 1	1	In general, the incidence of tumors in control and exposed rats was low or none. Both treated rats and controls showed reticulum cell sarcomas and mammary tumors. The study authors indicated that "numerous tumors characteristic of this strain were seen in all groups."
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were not explicitly specified (body weights at month 0 of treatment were shown graphically). No information on respiratory rate was reported, but this is not expected to substantially impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Data on attrition and/or health outcomes not related to exposure were not reported because there were not any significant differences among groups.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described (in minimal detail) and appear to be appropriate.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for all outcomes were presented by exposure group and sex. Measures of variation were not shown for all endpoints (hematology and clinical chemistry parameters).
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 20: **Animal toxicity evaluation results of Kasai et al 2009 for a 2-year cancer bioassay study on cancer, mortality, hepatic, renal, respiratory, hematological and immune, clinical chemistry/biochemical, nutrition and metabolic/adult exposure body weight, reproductive outcomes**

Study Citation:	Kasai, T; Kano, H; Umeda, Y; Sasaki, T; Ikawa, N; Nishizawa, T; Nagano, K; Arito, H; Nagashima, H; Fukushima, S (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats Inhalation Toxicology, 21(11), 889-897
Data Type:	2-year cancer bioassay
HERO ID:	193803

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 its identity was verified by analytical methods.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test chemical was reported as reagent grade (greater than 99% pure) and purity was also evaluated by the laboratory via gas chromatography-mass spectrometry (GC-MS). I downgraded this to medium because all seven lots tested were found to contain butylhydroxytoluene (avg level of 4.6 ppm [w/w]) by GC-MS, although no peak corresponding to this substance was found in air samples collected from the inhalation chamber.
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	Not applicable - Positive control group is not indicated by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The animals were divided by stratified randomization into body weight-matched groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance was found to be stable throughout the 7-month period of storage, as determined by gas chromatography. The methods and equipment used to generate the test substance were appropriate.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported and were consistent among the groups. However, I downgraded this to medium because the report does not specifically state that exposures occurred at the same time of day for all animals.

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Study Citation:	Kasai, T; Kano, H; Umeda, Y; Sasaki, T; Ikawa, N; Nishizawa, T; Nagano, K; Arito, H; Nagashima, H; Fukushima, S (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats Inhalation Toxicology, 21(11), 889-897
Data Type:	2-year cancer bioassay
HERO ID:	193803

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Actual vapor concentrations in the exposure chambers were measured and mean concentrations over the exposure period were reported (shown in Figure 1 of the study report).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and were appropriate for this type of study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified and adequate for the purpose of this study.
	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	Most of the test animal characteristics were reported (species, strain, sex, age, starting body weight); however, health status at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and were adequate and consistent among the groups and controls.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The outcome assessment protocol was reported; however, the descriptions of each outcome methodology do not specifically state that some outcomes (e.g., urine, blood) were sampled at the same time/day for all 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 (e.g., number of animals per group was adequate for the study type).

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Study Citation:	Kasai, T; Kano, H; Umeda, Y; Sasaki, T; Ikawa, N; Nishizawa, T; Nagano, K; Arito, H; Nagashima, H; Fukushima, S (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats Inhalation Toxicology, 21(11), 889-897
Data Type:	2-year cancer bioassay
HERO ID:	193803

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes to which blinding would be required were included and automated techniques (e.g., for blood biochemical analysis) were used for blood biochemical analysis. Histopathology examination results were not described as a re-evaluation so I considered this metric N/A.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences in initial weight, or food or water intake. However, this substance is considered an irritant (addressed in Discussion on p. 895, e.g., see citation Boatman & Knaak, 2001); however, respiratory rate measurement was not reported and this study, so I downgraded this metric rating to Low.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No indications of attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The statistical methods were clearly described and appropriate for the data set.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were shown for each exposure group. However, severity scores were not presented for histopathological changes that were observed in this study (e.g., pre- and non-neoplastic changes in Table 3) so I downgraded the score to medium.
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 21: **Animal toxicity evaluation results of Argus et al 1973 for a carcinogenicity-liver (dose response), electron microscopy study on cancer outcomes**

Study Citation:	Argus, MF; Sohal, RS; Bryant, GM; Hoch-Ligeti, C; Arcos, JC (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity European Journal of Cancer, 9(4), 237-243
Data Type:	Carcinogenicity-liver (dose response), electron microscopy
HERO ID:	62912

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Identified by name and source same as Argus et al., 1965 , which limits uncertainties
	Metric 2:	Test Substance Source	Medium	× 1	2	Source reported but no additional details were reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	There were no apparent differences in the concurrent control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric was not applicable.
	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	Solutions were prepared fresh daily in drinking water.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	There were no apparent inconsistencies in exposure administration.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported along with average fluid consumption
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Duration was provided
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were appropriate
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, age, initial body weight range, and source were reported. The health status of the animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if adequate.

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Study Citation:	Argus, MF; Sohal, RS; Bryant, GM; Hoch-Ligeti, C; Arcos, JC (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity European Journal of Cancer, 9(4), 237-243
Data Type:	Carcinogenicity-liver (dose response), electron microscopy
HERO ID:	62912

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals ranged from 28 to 32, but the group(s) that had less than 30 animals (slightly lower than cancer bioassay) was not specified.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Limited details in outcome assessment methodology was provided.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is inferred that outcome assessment was consistent.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were assessed.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	The biological responses of the control animals in the dose response study were not reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Details were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were not reported
	Metric 24:	Reporting of Data	Low	× 2	6	Data were described in the text, and descriptive tumor characteristics were not distinguished among groups. Effective tumor doses were reported
Overall Quality Determination‡			~~Unacceptable~~** ⟶ Low§		~~1.9~~	
Extracted			Yes			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

§ Evaluator's explanation for rating change: "The study would be upgraded because a description of the tumors observed was provided which is informative. Also, effective tumor doses were provided."

Table 22: **Animal toxicity evaluation results of Jbrc et al 1998 for a cancer bioassay and non-neoplastic lesions study on cancer, renal, hepatic, respiratory outcomes**

Study Citation:	JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water)					
Data Type:	Cancer bioassay and non-neoplastic lesions					
HERO ID:	196240					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, structure, and CASRN
	Metric 2:	Test Substance Source	Medium	× 1	2	Source was reported but no additional information.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test substance
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative control group was included
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation of animals was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance was administered in the drinking water, but additional details were not reported..
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were consistent
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, species, strain, sex, and age were reported. Starting body weight and health status were not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported.
	Metric 15:	Number per Group	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome methodology was appropriate and sensitive
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was appropriate
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						

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Study Citation:	JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water)
Data Type:	Cancer bioassay and non-neoplastic lesions
HERO ID:	196240

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no differences among groups unrelated to exposure
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were reported and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	Outcomes 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 23: **Animal toxicity evaluation results of Kano et al 2009 for a 2-year cancer bioassay study on cancer outcomes**

Study Citation:	Kano, H; Umeda, Y; Kasai, T; Sasaki, T; Matsumoto, M; Yamazaki, K; Nagano, K; Arito, H; Fukushima, S (2009). Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years Food and Chemical Toxicology, 47(11), 2776-2784
Data Type:	2-year cancer bioassay
HERO ID:	594539

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by CASRN and each lot analyzed by IR and GC.
	Metric 2:	Test Substance Source	High	× 1	1	Obtained from manufacturer.
	Metric 3:	Test Substance Purity	High	× 1	1	>99% pure; confirmed by GC
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Adequately reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not indicated for study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Stratified randomization; matched by body weight
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Adequately reported; prepared twice per week and stable at 4 days post-preparation.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Drinking water available to all animals ad libitum
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Data provided on water consumption; no difference across groups.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Consistent with test guideline for study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Highest dose chosen so as not to exceed the MTD.
	Metric 12:	Exposure Route and Method	High	× 1	1	Adequately reported. Consistent with test guideline for study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Adequately reported. Consistent with test guidelines for study type.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Adequately reported. Consistent with test guidelines for study type.
	Metric 15:	Number per Group	High	× 1	1	50/sex/group; consistent with test guidelines for study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Consistent with test guidelines for study type.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No anomalies reported.
	Metric 18:	Sampling Adequacy	High	× 1	1	Consistent with test guidelines for study type.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Not addressed.

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Study Citation:	Kano, H; Umeda, Y; Kasai, T; Sasaki, T; Matsumoto, M; Yamazaki, K; Nagano, K; Arito, H; Fukushima, S (2009). Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years Food and Chemical Toxicology, 47(11), 2776-2784
Data Type:	2-year cancer bioassay
HERO ID:	594539

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20: Negative Control Response	High	× 1	1	Adequately reported; no unusual results.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	High	× 2	2	Body-weight matching; no difference in food/water consumption.
	Metric 22: Health Outcomes Unrelated to Exposure	High	× 1	1	Attrition was related to exposure.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Appropriate methods chosen; adequately reported.
	Metric 24: Reporting of Data	High	× 2	2	Multiple data tables summarize all endpoints.
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

5 Developmental

Table 24: **Animal toxicity evaluation results of Giavini et al 1985 for a developmental-fetal effects study on growth (early life) and development outcomes**

Study Citation:	Giavini, E; Vismara, C; Broccia, ML (1985). Teratogenesis study of dioxane in rats Toxicology Letters, 26(1), 85-88					
Data Type:	Developmental-fetal effects					
HERO ID:	62924					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	The test substance was identified by name only
	Metric 2:	Test Substance Source	Medium	× 1	2	Source identified but no other details were reported. The omitted details are unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity and impurity identified; purity such that effects due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate controls used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Limited details on preparation and no details on storage were reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures administered consistently
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Details were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of exposure groups and spacing were appropriate
	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	Medium	× 2	4	The source, species, strain, initial body weight, and sex were reported. The age and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The humidity, light-dark cycle„ temperature, and availability of food and water were reported. The number of animals/cage was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The total number of animals per group were different, but a sufficient number of animals were available for statistical analysis.
Domain 5: Outcome Assessment						

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Study Citation:	Giavini, E; Vismara, C; Broccia, ML (1985). Teratogenesis study of dioxane in rats Toxicology Letters, 26(1), 85-88
Data Type:	Developmental-fetal effects
HERO ID:	62924

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was appropriate and sensitive.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed 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 was not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	There were no apparent issues with the biological response of the negative control group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were reported differences in maternal food consumption and body weight gain associated with treatment
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were reported or could be inferred .
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical tests were reported, but the parameters to which they were applied were not reported.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented for all outcomes by exposure groups.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

6 Genetic toxicity studies

Table 25: **Animal toxicity evaluation results of Goldsworthy et al 1991 for nasal epithelium DNA repair in rats**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Nasal Epithelium DNA repair
HERO ID:	62925

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (water).
	Metric 5:	Positive Controls	High	× 1	1	A positive control was utilized.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage of the test substance was not reported, but test substance administered in water and test substance is known to be soluble in water.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	A consistent concentration was administered in drinking water and gavage treatment was conducted consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentration (single concentration) administered in drinking water was reported. Additional single gavage doses were reported. No palatability issues were described, but body weights and water consumption were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration was reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest, but there was no justification for why the doses and spacing were selected.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Nasal Epithelium DNA repair
HERO ID:	62925

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per group is not specifically reported, but the footnote of Table 6 suggests that only two animals were used.
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 was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	An adequate number of slides and cells were evaluated.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	The study did not report on initial body weights or food/water intake during this drinking water study.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 26: **Animal toxicity evaluation results of Kitchin and Brown 1990 for acute rats study on liver DNA damage**

Study Citation:	K. T. Kitchin, J. L. Brown (1990). Is 1,4-dioxane a genotoxic carcinogen? Cancer Letters, 53(1,1), 67-71
Data Type:	Acute rat liver DNA damage
HERO ID:	62928

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: 1,4 dioxane, and mol wt.: 88.11
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from Aldrich Chem Co. Inc. Milwaukee, WI. No information reported on batch/lot number; however, the test substance is unlikely to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was highly pure: 99+% purity, no impurities were reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A corn oil control group was indicated in Table 1; however, the paper does not explicitly state that the test material was dissolved in corn oil or whether the corn oil controls were administered the same dose volume at the same time prior to sacrifice (this was assumed).
	Metric 5:	Positive Controls	Not Rated	NA	NA	NA: positive control was not necessary based on study type
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation of animals into study groups is not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Table 1 suggests that the test substance was prepared in corn oil (not explicitly stated). Test substance storage was not described; however, omission of these details are unlikely to have a substantial impact on results (only 2 doses were given 17 hours apart).
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Table 1 states oral admin., and indicates that dosing was administered 4 and 21 hours prior to sacrifice which occurred at consistently at 12:00. These details suggest a gavage route of exposure; however, the gavage volume was not reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Doses are reported in mg/kg: 0, 168, 840, 2550, 4200 mg/kg. the doses were given according to body weight (route not specified but assumed to be gavage as negative control is corn oil- common use in gavage and 2 single administrations indicate gavage as well)

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Study Citation: K. T. Kitchin, J. L. Brown (1990). Is 1,4-dioxane a genotoxic carcinogen? Cancer Letters, 53(1,1), 67-71
Data Type: Acute rat liver DNA damage
HERO ID: 62928

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Dosing was 2 single administrations at 21 and 4 hours prior to sacrifice. Cited previous literature indicating that a 4h timepoint was sufficient for DNA damage
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Doses were diluted from the acute rat oral LD50:100%, 60%, 20%, 4%
	Metric 12:	Exposure Route and Method	Low	× 1	3	Table 1 reports route as oral and the method is assumed to be gavage (corn oil as vehicle control, mg/kg dosing administered as single doses (2x), however it was not reported
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Female SD rats (CD strain) from Charles river laboratories (Raleigh NC) were 90 days old and acclimated for several weeks. Health status and starting BW was not reported. Animal is routinely used for outcome of interest
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	72 +/- 4 degrees F, 50 +/-10% humidity and 12 h light cycle 6am-6pm on, housed 3/ cage and acclimated for several weeks. Husbandry conditions were adequate and same for all dose groups
	Metric 15:	Number per Group	High	× 1	1	4-13 F rats/group reported in table 1. The number of animals per study group was appropriate for the study type and outcome analysis
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	DNA damage was reportedly done "as previously described" reference 8. Table 1 indicates that DNA damage was evaluated by alkaline elution which is a sensitive and appropriate method for detection of DNA damage, but few methodological details are provided
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent in protocol and time across all study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Details regarding blinding are not applicable for this study type as assessing subjective outcomes was not necessary
	Metric 20:	Negative Control Response	High	× 1	1	The biological response in the negative control group was adequate
Domain 6: Confounding / Variable Control						

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Study Citation:	K. T. Kitchin, J. L. Brown (1990). Is 1,4-dioxane a genotoxic carcinogen? Cancer Letters, 53(1,1), 67-71
Data Type:	Acute rat liver DNA damage
HERO ID:	62928

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial BW was not reported. The specific route was not reported (oral not further described) therefore it is not known if palatability influenced outcome and it was not reported. No body weight food or water consumption or clinical signs were reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical tests include an ANOVA followed by a student's t test of findings from the ANOVA; sufficient data were provided to allow for other statistical tests..
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data are reported in table 1 as mean +/- SEM with n reported below. Reported by dose group
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 27: **Animal toxicity evaluation results of Yoon et al 1985 for sex linked recessive lethal mutations in Drosophila study**

Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	1, 4, D sex linked recessive lethal in drosophila
HERO ID:	194373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Table 1 number 24 1,4 dioxane, CASRN:123-91-1 and structure included, MW was 88.12
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source is Fisher #785133 (in table 1). Lot number was not reported; however, the test substance is unlikely to vary in composition
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity reported in table 1: Labeled purity- "purified", analyzed purity- blank
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative concurrent controls were used. It was not reported if the negative controls were vehicle or untreated
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation of animals into study groups is not reported but may be included in the previous papers cited (Woodruff et al. 1984, Zimmering et al., 1984, or Valencia et al., 1985) for stock, mating schemes, protocols and methods.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared using water as the solvent. storage were not described but omission of these details is unlikely to have a substantial impact on results (3 day diet and injection).
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Protocols were from previously cited literature and were not reported in text.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Feeding dose reported in table 2 as 0, 35,000 ppm; injection doses are reported as 0, 50,000 ppm.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Feeding study duration was 3 days (assume continuous); while exact doses achieved could not be confirmed, injection was administered if no mutation occurred with dietary exposure
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Concentration was selected based on solubility, palatability, and toxicity (not further described). Single dose group for each route.

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Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	1, 4, D sex linked recessive lethal in drosophila
HERO ID:	194373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route is reported as oral dietary study and if no mutation are induced, the chemical is injected. It was not reported whether diet was prepared daily to account for volatility
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Drosophila stocks and mating schemes were not reported in text, but cited in (Woodruff et al, 1984; Zimmering et al, 1984; Valencia et al, 1985). Canton-S males were mated in 3 consecutive harems with Basc females over 7 days
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.in text.
	Metric 15:	Number per Group	High	× 1	1	At least 20 F2 Basc males (or Basc/+ females) were examined. Statistical analysis (power) was not reported but number is consistent with the study type
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Testing protocols and experimental methods were cited in (Woodruff et al, 1984; Zimmering et al, 1984; Valencia et al, 1985).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent in protocol and time across all study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Details regarding sampling adequacy are not applicable for this study type
	Metric 19:	Blinding of Assessors	High	× 1	1	A blind test for induction of SLRLs
	Metric 20:	Negative Control Response	High	× 1	1	The biological response in the negative control group was adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Palatability was reported to be part of the dose selection process but is not further described.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not conducted, however, sufficient data were provided to allow for other statistical tests..
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data are reported in table 2 by dose group and summary data are reported in table 4
Overall Quality Determination‡			Medium		1.8	

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Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	1, 4, D sex linked recessive lethal in drosophila
HERO ID:	194373

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 28: **Animal toxicity evaluation results of Kurl et al 1981 for RNA synthesis in rat liver study**

Study Citation:	R. N. Kurl, L. Poellinger, J. Lund, J. A. Gustafsson (1981). Effects of dioxane on RNA synthesis in the rat liver Archives of Toxicology, 49(1,1), 29-33					
Data Type:	RNA synthesis in rat liver					
HERO ID:	195054					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was referred to as p-dioxane (1,4-dioxane).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (saline injection).
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not necessary for the endpoint measured in this study, (endogenous RNA polymerase activity in the liver).
	Metric 6:	Randomized Allocation	Low	× 1	3	The animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported, but storage of the test substance was not reported (single dose administered).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Single doses were reported as mg/rat and body weight was reported as a range (180-200 g).
	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	Justification was not provided for the selection of dose levels; however, the selected doses produced a range of responses
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal and health status were not reported. The age, range of starting body weights, strain, and sex of the test animal were reported.

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Study Citation:	R. N. Kurl, L. Poellinger, J. Lund, J. A. Gustafsson (1981). Effects of dioxane on RNA synthesis in the rat liver Archives of Toxicology, 49(1,1), 29-33
Data Type:	RNA synthesis in rat liver
HERO ID:	195054

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	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this endpoint (n = 6).
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	Number of technical replicates per liver was not reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 20:	Negative Control Response	High	× 1	1	No response was observed in the negative controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among starting body weights were reported. Food and water consumption were not reported, but this is appropriate for a study of this type (single dose administered; outcome measured up to 48 hr later).
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Mean values were reported as mean % of control for 6 rats; however, variance was not given and no statistical analysis was performed.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and all groups.
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

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

⋆ MWF = Metric Weighting Factor

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

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

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

where High =≥ 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 29: **Animal toxicity evaluation results for Mcfee et al 1994 for mice bone marrow micronucleus assay**

Study Citation:	A. F. Mcfee, M. G. Abbott, D. K. Gulati, M. D. Shelby (1994). Results of mouse bone marrow micronucleus studies on 1,4-dioxane Mutation Research, 322(2,2), 145-148
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195060

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance is referred to as 1,4-Dioxane and CASRN is correct.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance is not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were injected with saline.
	Metric 5:	Positive Controls	High	× 1	1	Positive controls were injected with mitomycin C and a positive response was observed.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Allocation methods of the study animals were not reported. However, two laboratories carried out two trials each, following the same protocol, and it can be assumed that the results from each location are sufficiently independent of each other.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was briefly reported (dissolved in PBS 2 hr prior to treatment), but the storage of the test substance was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Injection volume and frequency were consistent across exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	There was no ambiguity in the administered doses.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure regimen was appropriate for this endpoint (daily injections for 1 or 3 days with samples obtained 24-48 hr after last treatment).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of exposure groups and dose spacing was considered adequate (500, 1000, 2000 mg/kg).
	Metric 12:	Exposure Route and Method	High	× 1	1	Intraperitoneal injection is an appropriate route of administration for the test substance.
Domain 4: Test Organism						

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Study Citation:	A. F. Mcfee, M. G. Abbott, D. K. Gulati, M. D. Shelby (1994). Results of mouse bone marrow micronucleus studies on 1,4-dioxane Mutation Research, 322(2,2), 145-148
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195060

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal was not reported. It is also not clear if the source is identical for the two laboratories in this study. The starting body weights were also not reported, although it was included that all starting body weights were within 4 g of each other.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported for either of the two laboratories in this study.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and consistent across treatment groups.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate and sensitive.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across treatment groups and the two laboratories.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate. 2,000 polychromatic erythrocytes were scored per animal.
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that slides of bone marrow smears were coded and two observers scored separate slides for each animal.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control groups yielded negative responses.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Starting body weights were all within 4 g of each other, but the actual values of starting body weights were not reported. No food or water consumption data was included, but this is appropriate for this type of study.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	The authors report that no attrition or clinical signs of toxicity were observed in any treatment group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical tests used were appropriate for the data assuming that data were normally distributed; however, no test for normality was reported.
	Metric 24:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination[‡]			High		1.5	
Extracted			Yes			

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Study Citation:	A. F. Mcfee, M. G. Abbott, D. K. Gulati, M. D. Shelby (1994). Results of mouse bone marrow micronucleus studies on 1,4-dioxane Mutation Research, 322(2,2), 145-148
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195060

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 30: **Animal toxicity evaluation results of Mirkova 1994 for mice bone marrow micronucleus assay**

Study Citation:	E. T. Mirkova (1994). Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay Mutation Research, 322(2,2), 142-144
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195062

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reported to be "of analytical grade."
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative control groups were included, but it is not specified whether these animals were treated with vehicle (water) or left untreated.
	Metric 5:	Positive Controls	High	× 1	1	Appropriate concurrent positive control groups were included (cyclophosphamide oral gavage).
	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	Preparation of the test substance was reported. Storage of the test substance was not reported; however, the test solutions were prepared immediately prior to use (single dose administered).
	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	Low	× 2	6	The species, strain, and sex of the test animals were reported. The commercial source, starting body weight range, and ages were 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. T. Mirkova (1994). Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay Mutation Research, 322(2,2), 142-144
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195062

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 = 4-10).
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	Sampling was adequate for the outcome of interest (2,000 polychromatic erythrocytes per animal).
	Metric 19:	Blinding of Assessors	Low	× 1	3	The authors state that slides were prepared and assessed as described previously (Ashby and Mirkova 1987) but do not state specifically that slides were coded
	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	Low	× 2	6	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	One of 6 total BALB/c male mice was found dead at 24 h post-treatment after 5000 mg/kg 1,4-dioxane administration. This is in line with the 4-day mean lethal dose (MLD4) identified in a preliminary study, 4500 mg/kg. No other deaths or health outcomes were reported for any treatment group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately (one-tailed t-test) The raw data are provided, enabling an independent of the data if necessary.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported adequately.
Overall Quality Determination[‡]			High		1.5	
Extracted			Yes			

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Study Citation:	E. T. Mirkova (1994). Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay Mutation Research, 322(2,2), 142-144
Data Type:	Mouse bone marrow micronucleus assay
HERO ID:	195062

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 31: **Animal toxicity evaluation results for Miyagawa et al 1999 for DNA synthesis in rat liver study**

Study Citation:	M. Miyagawa, T. Shirotori, M. Tsuchitani, K. Yoshikawa (1999). Repeat-assessment of 1,4-dioxane in a rat-hepatocyte replicative DNA synthesis (RDS) test: Evidence for stimulus of hepatocyte proliferation Experimental and Toxicologic Pathology, 51(6,6), 555-558
Data Type:	DNA synthesis in rat liver
HERO ID:	195063

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used, but these animals were untreated rather than receiving a vehicle (corn oil) gavage.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not necessary based on endpoint and study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported, but the storage of the test substance was not reported (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was 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	Exposure frequency and duration were appropriate for this endpoint, as evidenced by the timecourse data presented.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of exposure groups and dose spacing were appropriate and justified for this endpoint (½, ¾, 1x, and 2x the maximum tolerated dose).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and duration were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal starting body weights were not reported. The test animal species, strain, sex, age, and commercial source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were reported to be consistent across treatment groups, but specific values for temperature, humidity, and light-dark cycle were not included.

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Study Citation:	M. Miyagawa, T. Shirotori, M. Tsuchitani, K. Yoshikawa (1999). Repeat-assessment of 1,4-dioxane in a rat-hepatocyte replicative DNA synthesis (RDS) test: Evidence for stimulus of hepatocyte proliferation Experimental and Toxicologic Pathology, 51(6,6), 555-558
Data Type:	DNA synthesis in rat liver
HERO ID:	195063

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate for these endpoints (n = 3-4).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (2,000 hepatocytes per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results for this endpoint ([3H]thymidine or BrdU labeling evaluated using fluorescence microscopy).
	Metric 20:	Negative Control Response	High	× 1	1	Negative responses were observed in negative control groups.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weights were not reported. Food and water consumption and respiratory rates were not reported, but this is appropriate for this study design.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No attrition or health outcomes were reported in any treatment group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	The data were analyzed by t-test, but no test for normality was reported.
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 32: **Animal toxicity evaluation results of Morita and Hayashi 1998 for mouse liver micronucleus assay**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Mouse liver micronucleus assay
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (saline gavage).
	Metric 5:	Positive Controls	High	× 1	1	Appropriate concurrent positive control groups were included (mitomycin C injection).
	Metric 6:	Randomized Allocation	High	× 1	1	Random allocation of animals to treatment groups was reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of the test substance was reported. Storage of the test substance was not reported; however, the test solutions were prepared immediately prior to use (single dose administered).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was 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 selected doses were in line with previous oral gavage studies (listed in Table 1) and produced a range of responses in the liver micronucleus assay.
	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, age, sex, starting body weight range, and commercial source was provided for the test animals.

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Mouse liver micronucleus assay
HERO ID:	195065

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were reported to be consistent across treatment groups, but specific values for temperature, humidity, and light-dark cycle were not included.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 4-5).
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	Sampling was adequate for the outcomes of interest (2,000 hepatocytes per animal).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Authors state that selection and scoring were according to published criteria Braithwaithe and Ashby 1988 (in which slides are coded) but the authors do not specifically state whether slides were coded in this study
	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	A range for initial body weights was reported. 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 attrition or health outcomes were reported in any treatment group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Kastenbaum & Bowman's table was used to compare percent micronucleus results. In addition, individual animal data are provided, enabling re-analysis using different statistical procedures if necessary.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported adequately.
Overall Quality Determination[‡]			High		1.1	
Extracted			Yes			

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Mouse liver micronucleus assay
HERO ID:	195065

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

Table 33: **Animal toxicity evaluation results for Tinwell and Ashby 1994 for bone marrow micronucleus assay in mice**

Study Citation:	H. Tinwell, J. Ashby (1994). Activity of 1,4-dioxane in mouse bone marrow micronucleus assays Mutation Research, 322(2,2), 148-150
Data Type:	Bone Marrow Micronucleus assay in Mouse
HERO ID:	195086

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number were not reported; however the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used; dosed with vehicle (distilled water)
	Metric 5:	Positive Controls	High	× 1	1	An appropriate concurrent positive control was used.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was described. The storage of the test substance was not reported; however, omission of these details are unlikely to have a substantial impact on the results (single dose study).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was 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	Exposure frequency and duration were appropriate for this endpoint; single oral dose
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	There was one exposure group per experiment, with 3 experiments; the administered dose levels were justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, and sex were reported (health status, and starting body weight were not reported). The commercial source or in-house colony was not specified; however, these details were noted to have been described previously in a related study (Ashby and Mirkova, 1987). The test species and strain were an appropriate animal model for the evaluation of this endpoint.

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Study Citation:	H. Tinwell, J. Ashby (1994). Activity of 1,4-dioxane in mouse bone marrow micronucleus assays Mutation Research, 322(2,2), 148-150
Data Type:	Bone Marrow Micronucleus assay in Mouse
HERO ID:	195086

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported; however, these details were noted to have been described previously in a related study (Ashby and Mirkova, 1987)
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate for the study type and endpoints (n = 3-8).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were minor differences in the outcome assessment protocol, but these uncertainties or limitations are unlikely to have substantial impact on results. The studies were performed during a transition from the use of the Giemsa stain to acridine orange stain for evaluating bone marrow smears.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (2,000 polychromatic erythrocytes per animal).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding was not specifically reported in this study; The authors state that slides were prepared as described previously (Tinwell and Ashby, 1989), but blinding/coding of slides is not described in that paper either.
	Metric 20:	Negative Control Response	Low	× 1	3	The biological responses of the negative control groups were reported; however, there were deficiencies regarding the control responses that may have a substantial impact on results. One control animal in experiment 3 had an elevated MPE frequency which affected the determination of biological significance of treated mice.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were not reported. Food and water consumption were not reported, but this is appropriate for this study design.
	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 (one sided students t-test).
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination[‡]			High		1.5	
Extracted			Yes			

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Study Citation:	H. Tinwell, J. Ashby (1994). Activity of 1,4-dioxane in mouse bone marrow micronucleus assays Mutation Research, 322(2,2), 148-150
Data Type:	Bone Marrow Micronucleus assay in Mouse
HERO ID:	195086

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 34: **Animal toxicity evaluation results of Morita 1994 for mouse peripheral blood micronucleus assay**

Study Citation:	T. Morita (1994). No clastogenicity of 1,4 dioxane as examined in the mouse peripheral blood micronucleus test Honyu Dobutsu Shiken Bunkakai Kaiho, 2 7-8
Data Type:	Mouse peripheral blood micronucleus assay
HERO ID:	196085

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source and lot number of the test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of the test substance was not reported; however, the commercial source and lot number were identified, making it potentially possible to obtain the purity of that lot. This is not expected to have adversely affected the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (saline).
	Metric 5:	Positive Controls	High	× 1	1	Appropriate concurrent positive control groups were included (mitomycin C) and a positive result was observed.
	Metric 6:	Randomized Allocation	High	× 1	1	Random allocation of animals to treatment groups was reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of the test substance was reported. Storage of the test substance was not reported; however, the test solutions were prepared immediately prior to use (single dose administered).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was 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 selected doses were based off the maximum tolerated dose (3200 mg/kg), determined in a preliminary study. The number of exposure groups and dose spacing were reported and 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						

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Study Citation: T. Morita (1994). No clastogenicity of 1,4 dioxane as examined in the mouse peripheral blood micronucleus test Honyu Dobutsu Shiken Bunkakai Kaiho, 2 7-8
Data Type: Mouse peripheral blood micronucleus assay
HERO ID: 196085

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, age, sex, starting body weight range, and commercial source was provided for the test animals. Health status was not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 5).
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	Sampling was adequate for the outcomes of interest (1,000 reticulocytes assessed per animal per timepoint).
	Metric 19:	Blinding of Assessors	Low	× 1	3	blinding of assessors was not reported
	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	A range for initial body weights was reported. 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	One mouse in the highest dose group died between 48 and 72 hr post-treatment. This is considered to be treatment-related, as the highest dose was selected based on the maximum tolerated dose, determined in a preliminary study. No attrition occurred in any other treatment group, and no adverse health outcomes or clinical signs of toxicity were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Kastenbaum & Bowman's table was used to compare percent micronucleus results. In addition, individual animal data are provided, enabling a potential reanalysis using a different statistical test.
	Metric 24:	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:	T. Morita (1994). No clastogenicity of 1,4 dioxane as examined in the mouse peripheral blood micronucleus test Honyu Dobutsu Shiken Bunkakai Kaiho, 2 7-8
Data Type:	Mouse peripheral blood micronucleus assay
HERO ID:	196085

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 35: **Animal toxicity evaluation results of Stott et al 1981 for in vivo DNA synthesis, alkylation and repair in rats**

Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300
Data Type:	In vivo DNA synthesis, alkylation and repair
HERO ID:	1937837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by established nomenclature as 1,4-dioxane.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source of radiolabeled and unlabeled 1,4-dioxane was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was >99% for unlabeled compound; radiochemical purity was >98%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were used for the DNA synthesis experiments (saline for acute gavage exposure; drinking water for repeated dose exposure); negative controls were not needed for the DNA alkylation or repair experiments.
	Metric 5:	Positive Controls	High	× 1	1	Dimethylnitrosamine was used as a positive control for the DNA alkylation and repair experiments and positive responses were observed.
	Metric 6:	Randomized Allocation	High	× 1	1	Computer randomization was used to asign animals to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test solutions were prepared in saline. Storage was not described; however DNA alkylation and repair assays (and acute DNA synthesis assays) were single dose experiments, suggesting that omission of these details are unlikely to have a substantial impact on the results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Nominal concentrations for gavage exposures were reported; Nominal concentration administered in drinking water was reported, but actual doses were not reported; water intake rates and body weights were not reported
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were appropriate for this study type and outcome(s) of interest.

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Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300
Data Type:	In vivo DNA synthesis, alkylation and repair
HERO ID:	1937837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Doses were selected based on previous carcinogenicity studies (i.e., tumorigenic and non tumorigenic doses). 3 doses were used for acute studies of DNA synthesis; however only two doses were used for repeated dose exposures and a single high dose was used for DNA alkylation and repair assays.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage/drinking water administration is appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and starting body weight were reported (age and health status were not reported). Animals were obtained from a commercial laboratory.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	The study reports that rats were housed in "environmentally controlled animal holding rooms" but details of husbandry conditions were not sufficiently reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported and was low (4-6/group) for the DNA synthesis and repair assays. Only 2 animals were used to evaluate DNA alkylation.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methods were well described and appropriate and sensitive for the outcomes of interest; scintillation counting methodology for evaluating DNA repair is relatively insensitive.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling for the outcome of interest was reported and adequate
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the outcomes of interest (no subjective outcomes).
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported for DNA content and DNA synthesis following acute and repeated dose studies; relevant positive and negative control responses were reported for DNA repair.
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 study group. These deficiencies are likely to affect the results of the repeat dose DNA synthesis assay (11 week drinking water exposure).

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Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300
Data Type:	In vivo DNA synthesis, alkylation and repair
HERO ID:	1937837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group .
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for in vivo dataset(s) (Dunnett's t test).
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all in vivo outcomes by exposure group (mean +/1 SD).
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 36: **Animal toxicity evaluation results of Fujioka et al 2019 for in vivo mutations in rats**

Study Citation:	M. Gi, M. Fujioka, A. Kakehashi, T. Okuno, K. Masumura, T. Nohmi, M. Matsumoto, M. Omori, H. Wanibuchi, S. Fukushima (2018). In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats Archives of Toxicology, 92(10,10), 3207-3221
Data Type:	16-week drinking water study in F344 rats) in vivo mutation assay
HERO ID:	5029473

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number was not reported; however the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reported (> 99.9%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were tested (untreated drinking water) for all 3 experiments.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups for any of the three experiments.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance was dissolved in drinking water (not further described). Storage of the test substance was not reported and exposure was for 16 weeks.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups in all three experiments.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentration were reported without ambiguity. Concentrations reported in ppm drinking water. Measured water intake and 1,4-dioxane intake was reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Though the study authors did not justify the number of exposure groups or concentration spacing, the number of exposure groups and spacing of exposure levels appear to be adequate to show results relevant to the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.

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Study Citation:	M. Gi, M. Fujioka, A. Kakehashi, T. Okuno, K. Masumura, T. Nohmi, M. Matsumoto, M. Omori, H. Wanibuchi, S. Fukushima (2018). In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats Archives of Toxicology, 92(10,10), 3207-3221
Data Type:	16-week drinking water study in F344 rats) in vivo mutation assay
HERO ID:	5029473

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported while health status and starting body weight were not. It was noted that body weight was measured weekly. The test animal was from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of this endpoint. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and were adequate.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported; while slightly lower than typical for subchronic studies for some endpoints (N=5-8), it was sufficient for statistical analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently for all three experiments.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Automated procedures; details referenced in another publication.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were not reported; though drinking water and food consumption was reported. These minor uncertainties are unlikely to have a substantial impact on results. There were no other confounding variables noted in the study.
	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	All data were adequately reported.

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Study Citation:	M. Gi, M. Fujioka, A. Kakehashi, T. Okuno, K. Masumura, T. Nohmi, M. Matsumoto, M. Omori, H. Wanibuchi, S. Fukushima (2018). In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats Archives of Toxicology, 92(10,10), 3207-3221
Data Type:	16-week drinking water study in F344 rats) in vivo mutation assay
HERO ID:	5029473

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 =≥ 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 37: **In vitro evaluation results of Sina 1983 for mutagenesis in rat hepatocyte assay**

Study Citation:	J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391
Data Type:	DNA damage (SSB) in rat hepatocytes for 1,4-dioxane
HERO ID:	7323

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	Metric 2:	Test Substance Source	Medium	× 1	2	The commercial source of the test substance was identified, but lot number was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were included. It was not specified whether the negative controls were treated with water, DMSO, or left untreated.
	Metric 5:	Positive Controls	High	× 2	2	Dimethylnitrosamine was utilized as a positive control in each assay. Positive results were obtained from positive control groups. This compound requires metabolic activation and was also utilized as a validation of hepatocyte metabolism.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well-described.
	Metric 7:	Standards for Tests	High	× 1	1	The QC criteria were adequate to demonstrate validity, acceptability, and reliability of this test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance was reported. The storage of the test substance was not reported (single dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (3 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups and dose spacing was reported and appropriate.
	Metric 13:	Metabolic Activation	High	× 1	1	This assay did not include an exogenous metabolic activation step, as the cells used were primary rat hepatocytes.
Domain 4: Test Model						

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Study Citation:	J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391
Data Type:	DNA damage (SSB) in rat hepatocytes for 1,4-dioxane
HERO ID:	7323

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The identity and origin of the test model were reported. No additional information was provided.
	Metric 15:	Number per Group	Low	× 1	3	The number of plates independently treated with 1,4-dioxane is not specified (although 2 replicates/plate was indicated). This may suggest the use of a single culture per concentration. .
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	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	High	× 1	1	Statistical analysis was not conducted. A three-fold increase in DNA single-strand breaks over negative controls was considered to be a positive result. Raw data are available for statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria (DNA single-strand breaks) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The cytotoxicity of 1,4-dioxane was measured by trypan blue dye exclusion for all doses and by release of glutamate-oxaloacetate transaminase (GOT) from the cells at the two lowest doses. The methods were adequately described for each cytotoxicity assay.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391
Data Type:	DNA damage (SSB) in rat hepatocytes for 1,4-dioxane
HERO ID:	7323

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 38: **In vitro evaluation results of Galloway et al 1987 for chromosomal aberration study in Chinese hamster ovary cells**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro chromosomal aberration
HERO ID:	7768

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

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number of cells/dose (100) was reported and is slightly less than appropriate.
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring; slides were coded for scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

Table 39: **In vitro evaluation results of Galloway et al 1987 for sister chromatid exchanges study in Chinese hamster ovary cells**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro SCE
HERO ID:	7768

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

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro SCE
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of cells/dose was reported and is appropriate (50 cells/dose).
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	1,4-Dioxane in vitro SCE
HERO ID:	7768

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

Table 40: **In vitro evaluation results of Haworth et al 1983 forbacterial reverse mutation study**

Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for 1,4-dioxane
HERO ID:	28947

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported, including manufacturer lot number.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reported to be "Purified" according to the manufacturer label.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported and appropriate for different strains with and without metabolic activation. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on solubility limits or cytotoxicity. The number of exposure groups and dose spacing was reported and appropriate for this assay (100, 333.3, 1000, 3333.3, or 10000 µg/plate).

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Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for 1,4-dioxane
HERO ID:	28947

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. It was noted that the cultures were "routinely checked for genetic integrity as recommended by Ames et al. (1975)."
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary; however, the identity of each test substance assessed in this study was coded and not known to the assessors.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	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	High	× 1	1	A positive result was defined as a "reproducible, dose-related increase, whether it be twofold over background or not." Therefore, no statistical analysis was reported directly in the study; however, this is appropriate for this study design. Raw data are provided and could be analyzed independently.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with current standards.

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Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for 1,4-dioxane
HERO ID:	28947

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	High	× 1	1	A dose-setting experiment was conducted to assess cytotoxicity levels (viability, reduced numbers of colonies). If toxicity was observed in the preliminary experiment, the doses for the mutagenicity assay were selected so that the highest dose exhibited some degree of toxicity.
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡		High		1.1	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 41: **In vitro evaluation results of Goldsworthy et al 1991 for carcinogenicity in rat nasal epithelial cells and hepatocytes study**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Goldsworthy et al. 1991 in vitro hepatocyte DNA repair
HERO ID:	62925

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent media control was utilized.
	Metric 5:	Positive Controls	High	× 2	2	Two positive control groups were included in this study (2-Acetylaminofluorene dissolved in DMSO and dimethylnitrosamine).
	Metric 6:	Assay Procedures	Medium	× 1	2	Details on duration of cell incubation, medium, and use of a radioactive nucleoside were reported. Other details on test conditions are not reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage of the test substance was not reported, but information on solubility of test substance suggests unlikely impact on results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Cells were exposed in same culture medium for consistent lengths of time.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentrations were reported as point estimates.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for this study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and concentration spacing were adequate to evaluate a dose-response.
	Metric 13:	Metabolic Activation	High	× 1	1	Some groups included hepatocytes collected from rats pretreated with test substance to provide the opportunity for enzyme induction.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model and descriptive information were reported and appropriate.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Goldsworthy et al. 1991 in vitro hepatocyte DNA repair
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per group were reported and appropriate.
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	Details of the outcome assessment were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Adequate sampling (25 cells scored for each of 3 slides).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The outcome assessment relied on quantitative autoradiography. Blinding is not a concern in this study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences in test design and procedures were reported that would significantly influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were clearly described and presented.
	Metric 23:	Data Interpretation	High	× 2	2	The study authors described the evaluation criteria for the test and noted these were consistent with the cited standard protocol.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The methods of measurement were not fully described, but signs of toxicity were noted in the data table.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Goldsworthy et al. 1991 in vitro hepatocyte DNA repair
HERO ID:	62925

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 42: **In vitro evaluation results for Woo et al 1977 for DNA binding assay study**

Study Citation:	Y. T. Woo, M. F. Argus, J. C. Arcos (1977). Tissue and subcellular distribution of 3H-dioxane in the rat and apparent lack of microsome-catalyzed covalent binding in the target tissue Life Sciences, 21(10,10), 1447-1456
Data Type:	DNA binding assay
HERO ID:	62950

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as p-dioxane (1,4-dioxane). The dioxane was tritiated to trace radioactivity and referred to as 3H-dioxane throughout the study.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reported to be "of analytical or reagent grade." The purity was not reported, but this is not considered to have affected the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The negative control conditions for this experiment were the complete test system less the microsomes or NADPH system.
	Metric 5:	Positive Controls	High	× 2	2	Benzo[a]pyrene was included under the same conditions as a positive control. Positive results were observed under the positive control conditions.
	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	Medium	× 1	2	The preparation of the test substance was briefly described. The storage of the test substance was not described.
	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	Doses were reported in terms of radioactivity of the 3H-dioxane (82 µCi). Doses in mg/kg-bw can be calculated based on radioactivity of the 3H-dioxane (8.6 Ci/mmole). Therefore, doses were reported adequately.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	There was only one exposure group, but the dose was considered adequate for the outcome of interest.

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Study Citation: Y. T. Woo, M. F. Argus, J. C. Arcos (1977). Tissue and subcellular distribution of 3H-dioxane in the rat and apparent lack of microsome-catalyzed covalent binding in the target tissue Life Sciences, 21(10,10), 1447-1456
Data Type: DNA binding assay
HERO ID: 62950

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	In cases where, primary liver cells were harvested from rats following pretreatment with inducers of microsomal mixed function oxidases (phenobarbital, 3-methylchloanthrene, PCBs)
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model, calf thymus DNA, was reported but no other details were provided.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The replicates per study group were not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The positive control, benzo[a]pyrene, was not tested under all conditions that the dioxane was tested under (excluded +cytosol condition). Otherwise, the outcome assessment was reported to be consistent.
	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 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	No statistics were provided, because it appears that n = 1 for all test conditions..
	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	Medium	× 2	4	Results were reported for 3H-dioxane treatment only (no results reported for 14C-dioxane treatment).
Overall Quality Determination‡			Unacceptable⋆⋆		1.3	
Extracted			No			

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Study Citation:	Y. T. Woo, M. F. Argus, J. C. Arcos (1977). Tissue and subcellular distribution of 3H-dioxane in the rat and apparent lack of microsome-catalyzed covalent binding in the target tissue Life Sciences, 21(10,10), 1447-1456
Data Type:	DNA binding assay
HERO ID:	62950

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 43: **In vitro evaluation results of Nestmann et al 1984 for Ames test study**

Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	1,4-D Ames test
HERO ID:	194339

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 ("p-dioxane") in the study, though it was not described in any detail other than as a component of the 2 fabric protectors being evaluated.
	Metric 2:	Test Substance Source	Medium	× 1	2	The specific source of 1,4-dioxane was not stated in the paper, though the authors noted that "standards were obtained from Fisher Scientific Co., Limited, and Aldrich Chemical Co." Lot numbers were provided for TCE from these 2 sources. It is assumed that a standard of 1,4-dioxane was obtained from these sources. However, the uncertainty regarding the source of the test substance is not likely to impact the results of the study.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade of test substance were not reported. However, GC and GC-MS analyses were described in detail.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A "no-dose" control (also referred to in the study as a "control (blank) chamber") was included in the study.
	Metric 5:	Positive Controls	Medium	× 2	4	Four positive controls were employed and results shown on data summary tables, though they were not discussed in the text.
	Metric 6:	Assay Procedures	Medium	× 1	2	Study authors cite methods described in Ames et al. (1975) and obtained the tester strains from the Ames lab. Study authors noted a test deviation (not incorporating test substances into the top agar but rather adding them to open Petri dishes in dessicators containing the culture dishes).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Study describes preparation and storage of gaseous standards of test substances and general preparation of liquid samples added to culture dishes (it is assumed that these include 1,4-dioxane).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.

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Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	1,4-D Ames test
HERO ID:	194339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Nominal concentrations and time-weighted average exposure levels were reported for each exposure group.; air concentrations were measured and reported analytically
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Incubation period was 24 hours exposure to test substance, followed by an additional 24 hours prior to scoring plates. The plate incorporation method requires a 48-72 hour exposure.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Only 2 of 5 Salmonella strains were exposed to test substance, and only 3 exposure concentrations were employed for 1,4-dioxane.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Use of common metabolic activation system was reported, though not described in much detail.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Study employed commonly used bacterial strains and reported their source, but cited Ames et al. (1975) for a detailed description of them.
	Metric 15:	Number per Group	Low	× 1	3	Study employed 2 replicates/strain of bacteria. Initial bacterial cell counts were not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology reported the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to mutagenicity assays
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable (no subjective outcomes were assessed)
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No potential confounding variables were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	data interpretation was limited to calculating means of duplicate plates; means were considered different from background if there was a two-fold differences but not statistical analysis was performed.

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Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	1,4-D Ames test
HERO ID:	194339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Low	× 2	6	plates were scored for mutant colonies and background rates, but details of scoring methods were not provided
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Study did not evaluate cytotoxicity.
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported as revertants/plate for each exposure group, but data are insufficient to perform any statistical analysis (the only data reported is the mean of the duplicate plates).
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 44: **In vitro evaluation results of Zimmermann et al 1985 (194343) for an aneuploidy study in Saccharomyces cerevisiae**

Study Citation:	Zimmermann, FK; Mayer, VW; Scheel, I; Resnick, MA (1985). Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in Saccharomyces cerevisiae Mutation Research, 149(3), 339-351					
Data Type:						
HERO ID:	194343					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Unacceptable	× 2	8	Test substance is not clearly identified (it was referred to as dioxane in the study).
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Test substance source was not reported.
	Metric 3:	Test Substance Purity	Unacceptable	× 1	4	Test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A negative control was used, but not described (identity for the experiment referred to as dioxane was not reported).
	Metric 5:	Positive Controls	High	× 2	2	A positive control was used.
	Metric 6:	Assay Procedures	Unacceptable	× 1	4	Assay methods were not reported for the study referred to as dioxane.
	Metric 7:	Standards for Tests	Unacceptable	× 1	4	QC criteria were not specifically reported for the dioxane study.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information was provided on the preparation and storage.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details were not reported for the dioxane study.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Exposure concentrations were reported for the study for dioxane.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was acceptable for this type of study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported. Spacing was acceptable.
	Metric 13:	Metabolic Activation	Unacceptable	× 1	4	No information was provided on metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (yeast) was reported and was acceptable for evaluating mutagenicity.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of replicates per group was not reported for the dioxane study.
Domain 5: Outcome Assessment						

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Study Citation: Zimmermann, FK; Mayer, VW; Scheel, I; Resnick, MA (1985). Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in Saccharomyces cerevisiae Mutation Research, 149(3), 339-351

Data Type:

HERO ID: 194343

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There were no evidence inconsistencies among the exposure groups; however, there were few descriptive details for the dioxane study.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Limitations were reported for sampling although the duration following treatment was reported.
	Metric 19:	Blinding of Assessors	Unacceptable	× 1	4	Blinding was not reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding variables reported but based on limited details confidence is low.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	No outcomes unrelated to exposure were reported, but based on limited details confidence is low.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Data were provided. Few/no details regarding conduct of statistical analysis was provided.
	Metric 23:	Data Interpretation	Low	× 2	6	There was indication that scoring and/or evaluation criteria were not consistent with current guidelines; however, few details were provided so confidence is low.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity was not defined or presented.
	Metric 25:	Reporting of Data	Low	× 2	6	Data that were presented were acceptable to demonstrate a negative result; however, few details were provided so confidence is low.
Overall Quality Determination‡			Unacceptable⋆⋆		3.5	
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 $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out 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: **Animal toxicity evaluation results of Goldsworthy et al 1991 for gavage study in rats on hepatocyte cell proliferation**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Gavage Study - Hepatocyte Cell Proliferation
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (water).
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control group was needed for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study notes that the test substance was administered in water and the test substance is known to be soluble in water. Storage conditions were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent gavage volumes were administered.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single dose) was reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single dose level was utilized, but this was considered adequate for evaluating hepatocyte cell replication at different time points compared to controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Gavage Study - Hepatocyte Cell Proliferation
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals in the exposed treatment groups was adequate for the outcome analysis (n = 5), but a smaller number of animals was included in the negative control group (n = 3) without reference to a historical dataset.
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 as consistent for all groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	The number of hepatocyte nuclei (n=2,000) from each liver section was adequate for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The outcome assessment relied on quantitative autoradiography. Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not report on initial body weights or food/water intake during this particular study, but this is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and 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.

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

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out 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 Mcgregor et al 1991 for mice lymph cell mutation assay study**

Study Citation:	D. B. Mcgregor, A. G. Brown, S. Howgate, D. Mcbride, C. Riach, W. J. Caspary (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals Environmental and Molecular Mutagenesis, 17(3,3), 196-219
Data Type:	1,4-D Mouse Lymph Cell Mutation Assay
HERO ID:	194381

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by established name, CASRN, and chemical structure.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified in the report.
	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	Fischer's medium without serum was used as a vehicle control.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were used; their response was appropriate (significant increase in mutation frequency).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	High	× 1	1	The paper followed quality control guidelines and response criteria described in Caspary 1988.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	General information regarding test substance preparation was included, but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information on exposure administration was reported and consistency of administration is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported for each of the trials.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration for each phase of the testing was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure groups and concentration spacing was based on initial toxicity testing and is considered adequate.
	Metric 13:	Metabolic Activation	High	× 1	1	Trials were run with and without metabolic activation. Preparation of S9 was described in detail.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was described and is appropriate.

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Study Citation:	D. B. Mcgregor, A. G. Brown, S. Howgate, D. Mcbride, C. Riach, W. J. Caspary (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals Environmental and Molecular Mutagenesis, 17(3,3), 196-219
Data Type:	1,4-D Mouse Lymph Cell Mutation Assay
HERO ID:	194381

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of cells/culture was reported, as well as the number of replicate cultures/exposure concentration. They are appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Automated colony counting was employed and chemicals were coded during the study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Authors reported that one of the positive control cultures was contaminated, but data from the remaining exposure replicates or groups were valid. The trial containing this culture was not reported in final results tables as it failed to meet quality control criteria of the study.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical evaluations were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity test was described by the study authors as the first step in evaluation, to determine the exposure concentrations for the test substance.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	D. B. Mcgregor, A. G. Brown, S. Howgate, D. Mcbride, C. Riach, W. J. Caspary (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals Environmental and Molecular Mutagenesis, 17(3,3), 196-219
Data Type:	1,4-D Mouse Lymph Cell Mutation Assay
HERO ID:	194381

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

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

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

Table 47: **In vitro evaluation results of Hellmér and Bolcsfoldi 1992 for DNA repair in E. coli study**

Study Citation:	L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
Data Type:	1,4-D in vitro DNA repair test in E. coli
HERO ID:	194717

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was not specifically reported, but it was noted that the chemicals tested were purchased from a commercial source. The product number and batch/lot number were also not reported; however, the material is not expected to vary in composition. The omitted details are unlikely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance were not reported. It was noted that all chemicals tested were of the highest purity obtainable.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Study authors report using a concurrent negative solvent control; however, the solvent used for 1,4-dioaxane was not specified. This limitation is unlikely to have a substantial impact on results.
	Metric 5:	Positive Controls	High	× 2	2	A positive control (4-nitroquinoline-N-oxide) was used for tests without S9 metabolic activation; no positive control was used for tests with the S9 metabolic activation.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described, but appear to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported; the solutions were made immediately before the experiment and did not need to be stored.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The highest concentration was reported; there were no effects at this concentration.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (1 day)

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Study Citation:	L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
Data Type:	1,4-D in vitro DNA repair test in E. coli
HERO ID:	194717

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups and dose/concentration spacing were justified by study authors (diluted in 7 half log steps or 2-fold dilution steps; but only the highest concentration was reported. The number of exposure concentrations is unclear; because there were no effects at the highest concentration, it is unlikely to have a substantial impact on results.
	Metric 13:	Metabolic Activation	High	× 1	1	Exposures were conducted in the presence and absence of a metabolic activation system. The source and method of preparation were reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and source were reported and appropriate for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	The volume of bacterial mix was reported. One plate per concentration was tested.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently for all three experiments.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables noted in the study.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variable unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical methods were described and appropriate for the dataset. It was noted that the confidence interval was determined according to the variance for each strain from a previous experiment; this data was not presented.
	Metric 23:	Data Interpretation	High	× 2	2	The scoring/evaluation criteria was reported (if the number of colonies was $<$ 2 standard deviations of the mean for the strains, the test was considered significant)

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Study Citation:	L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
Data Type:	1,4-D in vitro DNA repair test in E. coli
HERO ID:	194717

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity endpoints were not defined; however, there was no effect at the highest concentration tested. Cytotoxicity was not a factor in this study.
	Metric 25:	Reporting of Data	Medium	× 2	4	The data for the outcome was reported. The study was negative at the highest dose tested
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 48: **In vitro evaluation results of 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 1,4-dioxane
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 1,4-dioxane with the correct CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of 1,4-dioxane 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 1,4-dioxane 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 1,4-dioxane 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	Low	× 1	3	Details of 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	Low	× 1	3	Assay methods were cited to other publications, preparation and storage were not specified
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	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	Low	× 2	6	The assay procedures were described as "routine protocol" and cited in other references.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups and dose spacing were not reported. The assay procedures were described as "routine protocol" and cited in other references.

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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 1,4-dioxane
HERO ID:	194949

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
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	Low	× 1	3	The number of plates per treatment group was not reported. The assay procedures were described as "routine protocol" and cited in other references.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	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	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	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	Low	× 2	6	Effect is reported as positive or negative for each chemical, but specific data (ie specific rates of mutagenicity relative to background) are not provided

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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 1,4-dioxane
HERO ID:	194949

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 49: **In vitro evaluation results of Khudoley et al 1987 for bacterial reverse mutation study**

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

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as carbon tetrachloride with the correct CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of CCl4 was not reported. A subset of the 126 test substances were reported to have been synthesized at the home institution of the authors, so it can be assumed that the CCl4 was obtained from an unidentified commercial source.
	Metric 3:	Test Substance Purity	Low	× 1	3	It was reported that the "majority" of the 126 test substances were "chemically pure". The purity of CCl4 was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were included concurrently in study design.
	Metric 5:	Positive Controls	Low	× 2	6	Appropriate concurrent positive control test substances were included for each test condition with and without S9 activation. Positive control data were not reported.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay methods and procedures were cited to other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Assay methods were cited to other publications.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Assay methods were cited to other publications.
	Metric 10:	Reporting of Doses/Concentrations	Not Rated	NA	NA	Assay methods were cited to other publications.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The assay procedures were described as "routine protocol" and cited in other references.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	The number of exposure groups and dose spacing were not reported. The assay procedures were described as "routine protocol" and cited in other references.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.

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

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 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 CCl4
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 50:

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 51: **In vitro evaluation results of Morita and Hayashi 1998 for sister chromatid exchange**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Sister chromatid exchange
HERO ID:	195065

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (saline).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included with and without S9 activation (mitomycin C and benzo[a]pyrene, respectively). Positive control groups exhibited positive responses.
	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	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate and within the range of previous in vitro assays (provided in Table 1).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported. The concentration and exposure duration were appropriate. However, the concentration of S9 used for this assay was not specified.
Domain 4: Test Model						

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Sister chromatid exchange
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity, commercial source, doubling time, and karyotype features of the Chinese hamster ovary (CHO-K1) were identified. This strain is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	Each experimental condition was completed 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 100 well-spread metaphases (50/replicate) per experimental condition.
	Metric 19:	Blinding of Assessors	Low	× 1	3	The authors did not describe coding slides prior to scoring
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	Medium	× 1	2	Data were analyzed by ANOVA followed by Student's t-test. Student's t-test is not an appropriate test given the variety of experimental conditions (>2 groups). However, raw data is provided, which would allow for independent statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of sister chromatid exchanges per cell) is consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The assay was completed in conjunction with a measurement of cytotoxicity (trypan blue exclusion).
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported and include a range and standard deviation
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Sister chromatid exchange
HERO ID:	195065

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 52: **In vitro evaluation results of Morita and Hayashi 1998 for in vitro micronucleus study**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	In vitro micronucleus
HERO ID:	195065

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (saline).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included with and without S9 activation (mitomycin C and benzo[a]pyrene, respectively).
	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	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate and within the range of previous in vitro assays (provided in Table 1).
	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 used for this assay was not specified.
Domain 4: Test Model						

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	In vitro micronucleus
HERO ID:	195065

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14: Test Model	High	× 2	2	The identity, commercial source, doubling time, and karyotype features of the Chinese hamster ovary (CHO-K1) were identified. This strain is routinely used for the outcome of interest.
	Metric 15: Number per Group	Medium	× 1	2	Each experimental condition was completed 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 2,000 intact interphase cells per experimental condition.
	Metric 19: Blinding of Assessors	Low	× 1	3	Authors do not describe coding slides prior to characterization of micronucleus frequencies (as recommended in OECD test guidelines)
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	Medium	× 1	2	Statistical analysis was not conducted. A positive result was defined as 3x the solvent control value. Raw data were provided that would enable an independent statistical analysis.
	Metric 23: Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with micronuclei) was consistent with standards and guidelines.
	Metric 24: Cytotoxicity Data	High	× 1	1	The micronucleus assay was completed in conjunction with a measurement of cytotoxicity (trypan blue exclusion).
	Metric 25: Reporting of Data	Low	× 2	6	Data are reported as percent cells with micronuclei, averaging across duplicates (rather than providing information about variability across individual plates)
Overall Quality Determination‡		High		1.3	

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	In vitro micronucleus
HERO ID:	195065

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

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 53: **In vitro evaluation results of Morita and Hayashi 1998 for mouse liver micronucleus assay mouse lymphoma tk assay (MLA)**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Mouse lymphoma tk assay (MLA)
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (saline).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included with and without S9 activation (methyl methanesulfonate and cyclophosphamide). Positive control groups exhibited positive responses.
	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	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate and within the range of previous in vitro assays (provided in Table 1).
	Metric 13:	Metabolic Activation	High	× 1	1	The source, method of preparation, and concentration of the rat liver S9 fraction was reported.
Domain 4: Test Model						

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Mouse lymphoma tk assay (MLA)
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity, donor source, and doubling time of the mouse lymphoma cell line (L5178Y) were identified. This strain is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	Each assay was plated 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 this study type.
	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	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	High	× 1	1	The data were appropriately analyzed by pairwise comparison and linear trend tests.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was consistent with standards and guidelines.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The mouse lymphoma assay standard protocol includes a measurement to account for cytotoxicity (relative survival without selection agent).
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡			High		1.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 54: **In vitro evaluation results of Morita and Hayashi 1998 for chromosomal aberration study**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280					
Data Type:	Chromosomal aberration					
HERO ID:	195065					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included with and without S9 activation (mitomycin C and benzo[a]pyrene, respectively). Positive control groups exhibited positive responses.
	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	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate and within the range of previous in vitro assays (provided in Table 1).
	Metric 13:	Metabolic Activation	High	× 1	1	The source, method of preparation, and concentration of the rat liver S9 fraction was reported.
Domain 4: Test Model						

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Chromosomal aberration
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity, commercial source, doubling time, and karyotype features of the Chinese hamster ovary (CHO-K1) were identified. This strain is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	Each experimental condition was completed 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	Medium	× 2	4	The sampling was somewhat lacking at 200 well-spread metaphases per experimental condition
	Metric 19:	Blinding of Assessors	Low	× 1	3	The authors do not describe coding slides prior to scoring chromosomal aberrations
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	High	× 1	1	The data were appropriately analyzed by Fisher's exact test.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with chromosomal aberrations) was reported and consistent with standards and guidelines.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The chromosomal aberration assay was completed in conjunction with a measurement of cytotoxicity (trypan blue exclusion).
	Metric 25:	Reporting of Data	Medium	× 2	4	Data are reported in terms of aberrations per 100 cells (averaging across duplicate cultures) without any information on variability between the two duplicates
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Chromosomal aberration
HERO ID:	195065

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 55: **In vitro evaluation results of Morita and Hayashi 1998 for bacterial reverse mutation study**

Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Bacterial reverse mutation
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane with the correct CASRN.
	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 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included for each S. typhimurium and E. coli strain with and without S9 activation. Positive control groups exhibited positive responses.
	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	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing was within the range of previous in vitro assays (provided in Table 1) and additionally exceeded previous studies' dose ranges by an order of magnitude to confirm lack of mutagenicity.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
Domain 4: Test Model						

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Study Citation:	T. Morita, M. Hayashi (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay Environmental and Molecular Mutagenesis, 32(3,3), 269-280
Data Type:	Bacterial reverse mutation
HERO ID:	195065

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary.
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	High	× 1	1	The data were appropriately analyzed by Dunnett's test.
	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	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‡			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 56: **Animal toxicity evaluation results of Goldsworthy et al 1991 for gavage study in rats on hepatocyte DNA repair**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Gavage Study - Hepatocyte DNA Repair
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (water and corn oil).
	Metric 5:	Positive Controls	High	× 1	1	Two positive control groups were included in this study (2-Acetylaminofluorene in corn oil and dimethylnitrosamine in water).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage of the test substance was not reported, but test substance administered as single gavage dose.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent gavage volumes were administered.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration was reported (single dose) and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single dose level (the highest dose recommended for this specific assay) was utilized and considered adequate for evaluating changes in DNA repair activity compared to controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Gavage Study - Hepatocyte DNA Repair
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this endpoint (n = 3).
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	Medium	× 1	2	The study does not describe the timing of the outcome assessment, but the same protocol was applied for all groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	An adequate number of slides (n = 3) for each animal was evaluated. However, the number of cells counted for each slide (n = 25) is below what is required by the OECD guideline (n = 100). The study authors did not provide rationale for this difference, but cite a different standard protocol.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The outcome assessment relied on quantitative autoradiography. Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not report on initial body weights or food/water intake during this particular study, but this is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and 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 57: **In vitro evaluation results of Munoz et al 2002 (195066) for a meiotic non-disjunction in Drosophila study**

Study Citation:	Munoz, ER; Barnett, BM (2002). The rodent carcinogens 1,4-dioxane and thiourea induce meiotic non-disjunction in Drosophila melanogaster females Mutation Research, 517(1-2), 231-238
Data Type:	Meiotic non-disjuntion in Drosophila
HERO ID:	195066

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source identified by name.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was briefly reported and no storage information was reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and spacing were reported, but justification was not reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model and limited descriptive information were reported.
	Metric 15:	Number per Group	High	× 1	1	The number of flies used was reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design and procedures.

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Study Citation:	Munoz, ER; Barnett, BM (2002). The rodent carcinogens 1,4-dioxane and thiourea induce meiotic non-disjunction in Drosophila melanogaster females Mutation Research, 517(1-2), 231-238
Data Type:	Meiotic non-disjuntion in Drosophila
HERO ID:	195066

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate analysis conducted.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Data not required.
	Metric 25:	Reporting of Data	High	× 2	2	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 58: **In vitro evaluation results of Sheu et al 1988 for mammalian cell transformation**

Study Citation:	C. W. Sheu, F. M. Moreland, J. K. Lee, V. C. Dunkel (1988). In vitro BALB/3T3 cell transformation assay of nonoxynol-9 and 1,4-dioxane Environmental and Molecular Mutagenesis, 11(1,1), 41-48					
Data Type:	Mammalian cell transformation					
HERO ID:	195078					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as 1,4-dioxane.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported, including manufacturer lot number.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reported to be "certified ACS grade," but purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported ("3-MCA", or methylcholanthrene) and appropriate. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported; however, solutions were prepared immediately before administration (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	A dose level resulting in approximately 10% survival in a preliminary cytotoxicity assay was selected as the maximum dose in the transformation assay. The number of exposure groups and dose spacing was reported and appropriate for this assay (250, 500, 1000, or 2000 µg/mL).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 4: Test Model						

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Study Citation:	C. W. Sheu, F. M. Moreland, J. K. Lee, V. C. Dunkel (1988). In vitro BALB/3T3 cell transformation assay of nonoxynol-9 and 1,4-dioxane Environmental and Molecular Mutagenesis, 11(1,1), 41-48
Data Type:	Mammalian cell transformation
HERO ID:	195078

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity, donor source, and passage number of the cell line used here were identified, and this cell line is appropriate for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 20 dishes per treatment group.
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	High	× 1	1	It was reported that the identity of the dishes in each group were coded.
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	Medium	× 1	2	The proportion of dishes with foci were analyzed using Fisher's exact test. The mean numbers of foci per dish were analyzed using "a procedure described by Lehmann (1959)." It was stated that the data were assumed to have normal distributions, which may not be a valid assumption.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported and consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A dose-setting experiment was conducted to assess cytotoxicity levels (viability, reduced numbers of colonies). The doses for the mutagenicity assay were selected so that the highest dose exhibited approximately 10% relative survival. Relative survival was also assessed concurrently with the transformation assay.
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

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Study Citation:	C. W. Sheu, F. M. Moreland, J. K. Lee, V. C. Dunkel (1988). In vitro BALB/3T3 cell transformation assay of nonoxynol-9 and 1,4-dioxane Environmental and Molecular Mutagenesis, 11(1,1), 41-48
Data Type:	Mammalian cell transformation
HERO ID:	195078

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 report of Stott et al 1981 for bacterial reverse mutation study**

Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300					
Data Type:	Bacterial reverse mutation					
HERO ID:	1937837					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,4-Dioxane was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer of 1,4-dioxane was identified. A lot number was not given; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity was reported as >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used; however, it was not clear whether cultures were untrated or exposed to vehicle (saline).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used for each strain and positive responses were observed.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods were not fully described, but were described as consistent with Ames, 1975 and appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared in saline. Storage conditions were not reported but this omission is unlikely to have a substantial impact on 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	Concentrations were reported without ambiguity (as mg/plate).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Method details are not provided; cited as Ames et al. (1975) so duration is likely to be consistent with assay standard.
	Metric 12:	Exposure Route and Method	High	× 1	1	5 concentration levels over 2 orders of magnitude.
	Metric 13:	Metabolic Activation	High	× 1	1	The enzyme activating system (S9 mix) was a rat liver homogenate obtained from Arochlor 1254~induced animals.
Domain 4: Test Model						

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Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300
Data Type:	Bacterial reverse mutation
HERO ID:	1937837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Source of the Salmonella test strains was listed as TA 1535, 1537m 1538m 98, and 100.
	Metric 15:	Number per Group	High	× 1	1	Data presented as mean of 3 replicates.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method addressed the intended outcome(s) of interest (reverse mutation) and was sensitive for the outcome(s) 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 was not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported among study groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variable unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	authors presented results as the mean across three replicates +/- standard deviation; authors did not describe methods for test significance of effects across treatments, but sufficient data were provided to conduct an independent statistical analysis.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Data evaluation criteria cited to Ames et al. (1975).
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 25:	Reporting of Data	High	× 2	2	Exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	W. T. Stott, J. F. Quast, P. G. Watanabe (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat Toxicology and Applied Pharmacology, 60(2,2), 287-300
Data Type:	Bacterial reverse mutation
HERO ID:	1937837

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 Dow et al 1989 (4158028) for an unscheduled DNA synthesis-liver (p 248) study**

Study Citation:	Dow Chemical Company (1989). The evaluation of 1,3-hexachlorobutadiene and 1,4-dioxane in the rat hepatocyte unscheduled DNA synthesis assay
Data Type:	Unscheduled DNA synthesis-liver (p 248)
HERO ID:	4158028

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, molecular weight and formula, and structure.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source and lot number were reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were include.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive control were included.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported and were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric not applicable for the test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance formulation was reported, but time between preparation and use was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and concentration spaces were adequate to address the purpose of the study, but concentrations were not justified. .
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Primary cultures do not have to be treated in the presence and absence of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model strain, source, age, husbandry conditions, and primary culture preparations were described.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates (n=3) adequate 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.

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Study Citation:	Dow Chemical Company (1989). The evaluation of 1,3-hexachlorobutadiene and 1,4-dioxane in the rat hepatocyte unscheduled DNA synthesis assay
Data Type:	Unscheduled DNA synthesis-liver (p 248)
HERO ID:	4158028

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number of cells/culture counted (n=15 cells/slide) were less than the minimum of 50 cells/culture recommended by OSCPP guideline 870.5550, but were sufficient for analyses.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	OSCPP guideline 870.5550 recommends coding slides prior to counting cells, but it is not stated that slides were coded before counting.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design and procedures.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the replicates or groups unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were described and appropriate.
	Metric 23:	Data Interpretation	High	× 2	2	The requirements for an unequivocal positive result were reported.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The highest dose tested was cytotoxic which is the only cytotoxicity recommendation for the assay.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for all 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 61: **In vitro evaluation results of Dow et al 1989 (4158030) for a genotoxicity study in salmonella**

Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Genotoxicity-Salmonella (p. 262)
HERO ID:	4158030

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Identified only by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source only was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that any effects are the result of the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were used
	Metric 6:	Assay Procedures	Medium	× 1	2	Specific details were not reported, however, the test was conducted as described by Ames et al., 1975.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Formulation was reported, but time between preparation and use was not reported.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were inferred from the text as they were not stated for all concentrations groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Test conducted according to Ames et al. 1975- which states ~48-hour incubation.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and concentration spaces were not justified, but were sufficient to address the purpose of the study.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The type and source of system were reported, but some details (e.g., composition mix, volume in final culture, concentration, QC information) were not included.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported with no additional details.
	Metric 15:	Number per Group	High	× 1	1	Test conducted according to Ames et al. 1975-which states to use 0.1 mL of culture.
Domain 5: Outcome Assessment						

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Study Citation:	Dow Chemical Company (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat
Data Type:	Genotoxicity-Salmonella (p. 262)
HERO ID:	4158030

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Test conducted according to Ames et al. 1975-which states to count the colonies and provides spontaneous revertant colony counts for east strain.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no details reported or inferred that suggested that outcome assessment was not consistent.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The metric is not applicable.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables in test design or procedures.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the replicates unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No calculation methods were reported, but sufficient data were provided to conduct independent analysis.
	Metric 23:	Data Interpretation	Medium	× 2	4	The evaluation criteria were partially reported (i.e., increased in background reversion rate), but given the results, the omission does not impact the results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were partially defined (i.e., background lawn toxicity), but this is not detrimental to the results.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

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

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

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

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

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

Table 62: **Animal toxicity evaluation results of Goldsworthy et al 1991 for drinking water study in rats on hepatocyte DNA repair**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte DNA Repair
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (water and corn oil).
	Metric 5:	Positive Controls	High	× 1	1	Two positive control groups were included in this study (2-Acetylaminofluorene in corn oil and dimethylnitrosamine in water).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage of the test substance was not reported, but test substance administered in drinking water and test substance is known to be soluble in water.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent concentration added to drinking water provided to rats.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentrations administered in drinking water were reported. No palatability issues were described, but body weights and water consumption were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration was reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest, but there was no justification for why the doses and spacing were selected.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte DNA Repair
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this endpoint (n = 3).
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	Medium	× 1	2	The study does not describe the timing of the outcome assessment, but the same protocol was applied for all groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	An adequate number of slides (n = 3) for each animal was evaluated. However, the number of cells counted for each slide (n = 25) is below what is required by the OECD guideline (n = 100). The study authors did not provide rationale for this difference, but cite a different standard protocol.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The outcome assessment relied on quantitative autoradiography. Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not report on initial body weights or food/water intake during this drinking water study.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte DNA Repair
HERO ID:	62925

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 63: **Animal toxicity evaluation results of Goldsworthy et al 1991 for drinking water study in rats on hepatocyte cell proliferation**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte Cell Proliferation
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized (water).
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control group was needed for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study notes that the test substance was administered in water and the test substance is known to be soluble in water. Storage conditions were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The study reports that animals were continuously administered a consistent concentration in the drinking water.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentrations in drinking water were reported along with the total average water intake for the exposed animals and the control animals. No palatability issues were described, but daily water intake rates and body weights were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single dose) was reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single dose level was utilized, but this was considered adequate for evaluating hepatocyte cell replication at different time points compared to controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte Cell Proliferation
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals in the exposed treatment groups was adequate for the outcome analysis (n = 5), but a smaller number of animals was included in the negative control group (n = 3) without reference to a historical dataset.
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 as consistent for all groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	The number of hepatocyte nuclei (n=2,000) from each liver section was adequate for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The outcome assessment relied on quantitative autoradiography. Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not report on initial body weights or food/water intake for individual animals during this particular study, but this is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Drinking Water Study - Hepatocyte Cell Proliferation
HERO ID:	62925

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 64: **Animal toxicity evaluation results of Goldsworthy et al 1991 for nasal cell proliferation in rats**

Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9					
Data Type:	Nasal Cell Proliferation					
HERO ID:	62925					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as "1,4-dioxane".
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The batch/lot number was not reported, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance was reported to be of HPLC grade, 99.9% purity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were utilized. Materials and methods does not specify a control group, but footnote to Table 8 reports control responses.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control group was needed for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study notes that the test substance was administered in water and the test substance is known to be soluble in water. Storage conditions were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The study reports that animals were continuously administered a consistent concentration in the drinking water.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Nominal concentration administered in drinking water was reported, but actual doses were not reported. No palatability issues were described, but water intake rates and body weights were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration was reported (single dose) and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A single dose level was utilized, but this was considered adequate for evaluating cell replication at different time points compared to controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were reported.

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Study Citation:	T. L. Goldsworthy, T. M. Monticello, K. T. Morgan, E. Bermudez, D. M. Wilson, R. Jäckh,Butterworth BE (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes Archives of Toxicology, 65(1,1), 1-9
Data Type:	Nasal Cell Proliferation
HERO ID:	62925

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequate and consistent across control and exposed groups.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals/group was not reported.
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 as consistent for all groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling adequacy was adequate for the specific outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not a concern in this study.
	Metric 20:	Negative Control Response	High	× 1	1	The control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not report on initial body weights or food/water intake during this particular study, but this is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes or deaths were reported in the study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
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 =≥ 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

7 Mechanistic

Table 65: **In vitro evaluation results of Shah et al 2015 (3115011) for a hepatic CYP450 enzyme activity (metabolism) study**

Study Citation:	Shah, TS; Kamble, SH; Patil, PG; Iyer, KR (2015). Effect of water-miscible organic solvents on cyp450-mediated metoprolol and imipramine metabolism in rat liver microsomes Indian Journal of Pharmaceutical Sciences, 77(4), 382-390
Data Type:	Hepatic CYP450 enzyme activity (metabolism)
HERO ID:	3115011

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified by name only,
	Metric 2:	Test Substance Source	Medium	× 1	2	Source identified by name only.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were exposed.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric not applicable.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail and applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Formulation details were reported, but time between preparation and use was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations and reaction volumes were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and concentration spacing were not justified by the study authors, but were sufficient to address the purposes of the study.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric not applicable
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Microsomes were obtained from rats sacrificed from other experiments, and were characterized for CYP450 content.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates was reported and appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was conducted consistently.

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Study Citation:	Shah, TS; Kamble, SH; Patil, PG; Iyer, KR (2015). Effect of water-miscible organic solvents on cyp450-mediated metoprolol and imipramine metabolism in rat liver microsomes Indian Journal of Pharmaceutical Sciences, 77(4), 382-390
Data Type:	Hepatic CYP450 enzyme activity (metabolism)
HERO ID:	3115011

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The rats from which liver microsomes were collected were only described as having been part of other experiments. It is unclear if the rats were obtained from control groups or treated groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No reported differences among the study replicates or groups were observed and the test substance did not interfere with the assay.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Calculation methods were reported and appropriate and sufficient data were provided to conduct statistical analyses..
	Metric 23:	Data Interpretation	Not Rated	NA	NA	This metric scored not applicable to this study type.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric not applicable
	Metric 25:	Reporting of Data	High	× 2	2	Outcome data were reported in the text and in tabular and graphical formats.
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 66: **In vitro evaluation results of Patil et al 2015 for a CYP2e1 activity in liver microsomes study**

Study Citation:	Patil, PG; Kamble, SH; Shah, TS; Iyer, KR (2015). Effect of water miscible organic solvents on p-nitrophenol hydroxylase (CYP2E1) activity in rat liver microsomes Indian Journal of Pharmaceutical Sciences, 77(3), 283-289
Data Type:	CYP2E1 activity in liver microsomes
HERO ID:	3117721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance only identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source identified only.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not applicable.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric not applicable for this test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Formulation protocol was included, but time between preparation and use was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations and reaction volume amounts were well described.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and adequate.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, were appropriate to address the purposes of the study.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation not required for this assay.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was described and reported to be characterized for CYP450 content.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates was adequate for outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate for outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes assessed.
Domain 6: Confounding / Variable Control						

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Study Citation:	Patil, PG; Kamble, SH; Shah, TS; Iyer, KR (2015). Effect of water miscible organic solvents on p-nitrophenol hydroxylase (CYP2E1) activity in rat liver microsomes Indian Journal of Pharmaceutical Sciences, 77(3), 283-289
Data Type:	CYP2E1 activity in liver microsomes
HERO ID:	3117721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Livers from rats sacrificed from other experiments were used but no additional data on the rats (i.e., control or treated) were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the replicates unrelated to exposure, and the test substance did not interfere with the assay,
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Calculation methods were described and data were reported in which statistical analyses can be conducted.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Data evaluation criteria not required for this test.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not measured.
	Metric 25:	Reporting of Data	High	× 2	2	Outcome data were reported in the text and in tabular and graphical formats.
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.

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

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