United States
Environmental Protection Agency

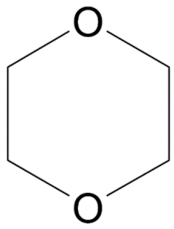
Office of Chemical Safety and
Pollution Prevention

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



June 2019

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1. Acute Toxicity Studies

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

Study reference:	**Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified definitively (by name).	High	1	2	2
	2. Test Substance Source	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.	Low	3	1	3
	3. Test Substance Purity	Test substance purity was reported as >99%.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	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.	Low	3	2	6
	5. Positive Controls	A concurrent positive control group is not necessary for this study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The study did not report how animals were allocated to study groups.	Low	3	1	3

Study reference:	Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	Details of exposure were reported for the most part and there was no indication to suggest that the exposures differed among the groups.	Medium	2	1	2
	9. Reporting of Doses/ Concentrations	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.	Low	3	2	6
	10. Exposure Frequency and Duration	Exposure duration and frequency were reported (4 hours, one exposure) and suitable for the study type and outcomes of interest.	High	1	1	1

Study reference:	Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and concentration spacing (1000 and 2000) ppm were relevant for the assessment.	High	1	1	1
	12. Exposure Route and Method	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.	Low	3	1	3
Test Organism	13. Test Animal Characteristics	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.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	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.	Low	3	1	3

Study reference:	Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	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.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology for this acute exposure study was limited to clinical chemistry/biochemistry parameters, specifically, serum enzyme analysis.	Low	3	2	6
	17. Consistency of Outcome Assessment	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.	High	1	1	1

Study reference:	Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	Details regarding sampling for the outcome(s) of interest were reported and acceptable for the outcomes of interest.	High	1	1	1
	19. Blinding of Assessors	No subjective endpoints were evaluated in this study.	Not Rated	NA	NA	NA
	20. Negative Control Response	Each rat served as its own control prior to exposure.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	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.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were described in sufficient detail and were appropriate for the data sets.	High	1	1	1
	24. Reporting of Data	Data presentation is incomplete. No data were presented for control groups.	Low	3	2	6
		Sum of scores:			**29**	**63**

Study reference:	Drew, R. T.,Patel, J. M.,Lin, F. N. (1978). Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination Toxicology and Applied Pharmacology, 45(3), 809-819. HERO ID: 67913					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:**		**2.1724**	**Overall Score: Nearest *:**	**2.2**
		Overall Quality Level:		**Medium**		

1.2. Animal toxicity evaluation results of Mattie et al 2012 for a 6-hour inhalation study - neuro study on neurological/behavioral outcomes

Study reference:	**Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)	High	1	2	2
	2. Test Substance Source	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)	Medium	2	1	2
	3. Test Substance Purity	>99% purity	High	1	1	1
Test Design	4. Negative and Vehicle Controls	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).	High	1	2	2
	5. Positive Controls	Positive control not required for study type (OPPTS 870.1300)	Not Rated	NA	NA	NA
	6. Randomized Allocation	Animals were "randomly selected for each exposure group".	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Vapor generation method was adequateely reported.	High	1	1	1
	8. Consistency of Exposure Administration	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.	Medium	2	1	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure duration consistent with cited guideline (OPPTS 870.1300)	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Five exposure groups plus concurrent controls were used. Exposure levels were based on levels in previous studies.	High	1	1	1
	12. Exposure Route and Method	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.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	High	1	2	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.	High	1	1	1
	15. Number per Group	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)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Clinical signs of neurotoxicity (autonomic effects, central nervous system effects, and reactivity to handling or sensory stimuli)	High	1	2	2
	17. Consistency of Outcome Assessment	Assessment identical across groups.	High	1	1	1
	18. Sampling Adequacy	Sampling consisted with cited guideline (OPPTS 870.1300)	High	1	1	1
	19. Blinding of Assessors	No reporting of blinding status of examiners during subjective assessments of clinical signs of neurotoxicity.	Unacceptable	4	1	4
	20. Negative Control Response	Results of clinical signs evaluations not reported for control or exposure group.	Unacceptable	4	1	4
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Medium	2	2	4

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	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.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	No mention of statistical analysis of clinical neurotoxicity evaluation (data not reported).	Unacceptable	4	1	4
	24. Reporting of Data	Results of clinical signs evaluations not reported for control or exposure group.	Unacceptable	4	2	8
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			30	50
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.6667**	**Overall Score (Rounded):**	**1.7[1]**
		Overall Quality Level:		**Unacceptable[1]**		

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

1.3. Animal toxicity evaluation results of Mattie et al 2012 for a 6-hour inhalation study - systemic effects study on hepatic, renal, irritation, respiratory, body weight outcomes

Study reference:	**Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)	High	1	2	2
	2. Test Substance Source	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)	Medium	2	1	2
	3. Test Substance Purity	>99% purity	High	1	1	1
Test Design	4. Negative and Vehicle Controls	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).	High	1	2	2
	5. Positive Controls	Positive control not required for study type (OPPTS 870.1300)	Not Rated	NA	NA	NA
	6. Randomized Allocation	Animals were "randomly selected for each exposure group".	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Vapor generation method was adequately reported.	High	1	1	1
	8. Consistency of Exposure Administration	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.	Medium	2	1	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure duration consistent with cited guideline (OPPTS 870.1300)	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Five exposure groups plus concurrent controls were used. Exposure levels were based on levels in previous studies.	High	1	1	1
	12. Exposure Route and Method	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.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	High	1	2	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.	High	1	1	1
	15. Number per Group	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)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	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	High	1	2	2
	17. Consistency of Outcome Assessment	Assessment identical across groups.	High	1	1	1
	18. Sampling Adequacy	Sampling consisted with cited guideline (OPPTS 870.1300)	High	1	1	1
	19. Blinding of Assessors	Only non-subjective outcomes and initial histopathological evaluations performed; blinding not necessary.	Not Rated	NA	NA	NA

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	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.	Medium	2	1	2
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	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.	High	1	1	1

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	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).	Medium	2	1	2
	24. Reporting of Data	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.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**39**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NA**	**Overall Score: Nearest *:**	**NA**
		Overall Quality Level:		**Medium**		

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: 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 from high. 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. Note: The original calculated score for this study was 1.4. This value is not presented above because the final rating was changed based on professional judgement.**					

2. Short-term Toxicity Tests

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

Study reference:	**Giavini, E.,Vismara, C.,Broccia, M. L. (1985). Teratogenesis study of dioxane in rats Toxicology Letters, 26(1), 85-88. HERO ID: 62924**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified by name only	Low	3	2	6
	2. Test Substance Source	Source identified but no other details were reported. The omitted details are unlikely to have a substantial impact on results.	Medium	2	1	2
	3. Test Substance Purity	Purity and impurity identified; purity such that effects due to test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Appropriate controls used.	High	1	2	2
	5. Positive Controls	This metric is not applicable.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The method of allocation was not reported.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Limited details on preparation and no details on storage were reported.	Medium	2	1	2
	8. Consistency of Exposure Administration	Exposures administered consistently	High	1	1	1
	9. Reporting of Doses/ Concentrations	Doses were reported without ambiguity.	High	1	2	2
	10. Exposure Frequency and Duration	Details were reported and appropriate.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Number of exposure groups and spacing were appropriate	High	1	1	1
	12. Exposure Route and Method	The route and method were suited to the test substance.	High	1	1	1

Study reference:	Giavini, E.,Vismara, C.,Broccia, M. L. (1985). Teratogenesis study of dioxane in rats Toxicology Letters, 26(1), 85-88. HERO ID: 62924					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The source, species, strain, initial body weight, and sex were reported. The age and health status were not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	The humidity, light-dark cycle,, temperature, and availability of food and water were reported. The number of animals/cage was not reported.	Medium	2	1	2
	15. Number per Group	The total number of animals per group were different, but a sufficient number of animals were available for statistical analysis.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was appropriate and sensitive.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate for the outcomes of interest.	High	1	1	1
	19. Blinding of Assessors	This metric was not applicable.	Not Rated	NA	NA	NA
	20. Negative Control Response	There were no apparent issues with the biological response of the negative control group.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were reported differences in maternal food consumption and body weight gain associated with treatment	Medium	2	2	4

Study reference:	Giavini, E.,Vismara, C.,Broccia, M. L. (1985). Teratogenesis study of dioxane in rats Toxicology Letters, 26(1), 85-88. HERO ID: 62924					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	No health outcomes unrelated to exposure were reported or could be inferred .	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical tests were reported, but the parameters to which they were applied were not reported.	Medium	2	1	2
	24. Reporting of Data	Data were presented for all outcomes by exposure groups.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**44**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.517**	**Overall Score: Nearest *:**	**1.5**
		Overall Quality Level:		**High**		

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

Study reference:	**Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance was identified definitively.	High	1	2	2
	2. Test Substance Source	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.	Low	3	1	3
	3. Test Substance Purity	Purity and grade were not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	A concurrent negative control group was tested and was appropriate.	High	1	2	2
	5. Positive Controls	A concurrent positive control group is not necessary for this study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	Animals were randomized and distributed into groups.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	Details of the exposure administration were reported and exposures were administered consistently across study groups.	High	1	1	1

Study reference:	Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/ Concentrations	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.	Low	3	2	6
	10. Exposure Frequency and Duration	The exposure frequency and duration of exposure were reported and were appropriate for this study type and the outcomes of interest.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	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.	High	1	1	1

Study reference:	Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	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.	Low	3	1	3
Test Organism	13. Test Animal Characteristics	The test animal species, strain, sex, age, and starting body weight were reported. Health status at the start of the study was not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	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.	Low	3	1	3
	15. Number per Group	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.	Medium	2	1	2

Study reference:	Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	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.	Medium	2	2	4
	17. Consistency of Outcome Assessment	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.	Low	3	1	3
	18. Sampling Adequacy	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).	Low	3	1	3

Study reference:	Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	19. Blinding of Assessors	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.	Low	3	1	3
	20. Negative Control Response	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.	Low	3	1	3
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6

Study reference:	Goldberg, M. E.,Johnson, H. E.,Pozzani, U. C.,Smyth, H. F., 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. HERO ID: 58035					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	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.	Low	3	1	3
	24. Reporting of Data	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).	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**67**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.2333**	**Overall Score: Nearest *:**	**2.2**
		Overall Quality Level:		**Medium**		

2.3. Animal toxicity evaluation results of Mattie et al 2012 for a 2-week inhalation study - neurological/behavioral, body weight outcomes

Study reference:	**Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)	High	1	2	2
	2. Test Substance Source	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)	Medium	2	1	2
	3. Test Substance Purity	>99% purity	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were exposed to clean air.	High	1	2	2
	5. Positive Controls	Positive control not required for study type (OECD 412)	Not Rated	NA	NA	NA
	6. Randomized Allocation	Animals were "randomly selected for each exposure group".	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Vapor generation method was adequately reported.	High	1	1	1
	8. Consistency of Exposure Administration	Exposure methods were consistent between groups.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure duration consistent with cited guideline (OECD 412)	High	1	1	1

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	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.	High	1	1	1
	12. Exposure Route and Method	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.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	High	1	2	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.	High	1	1	1
	15. Number per Group	16/sex/group; 8/sex sacrificed at end of exposure, 8/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group per sacrifice)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	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)	High	1	2	2
	17. Consistency of Outcome Assessment	Assessment identical across groups.	High	1	1	1
	18. Sampling Adequacy	Sampling consisted with cited guideline (OECD 412)	High	1	1	1
	19. Blinding of Assessors	No reporting of blinding status of examiners during subjective assessments of clinical signs of neurotoxicity.	Unacceptable	4	1	4
	20. Negative Control Response	Body weights and results of clinical signs evaluations were not reported for control or exposure group.	Unacceptable	4	1	4

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	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.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	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)	Unacceptable	4	1	4
	24. Reporting of Data	Body weights and results of clinical signs evaluations were not reported for control or exposure groups.	Unacceptable	4	2	8
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			30	51
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.7**	**Overall Score (Rounded):**	**1.7[1]**
		Overall Quality Level:		**Unacceptable[1]**		

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Footnote: **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, four of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**						

2.4. Animal toxicity evaluation results of Mattie et al 2012 for a 2-week inhalation study - systemic effects study on hepatic, renal, irritation, respiratory, hematological and clinical chemistry outcomes

Study reference:	**Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Clearly identified: 1,4-dioxane ((formula: C4H8O2); CAS # 123-91-1)	High	1	2	2
	2. Test Substance Source	Purchased from Sigma-Aldrich, Inc.. (batch no. not reported)	Medium	2	1	2
	3. Test Substance Purity	>99% purity	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were exposed to clean air.	High	1	2	2
	5. Positive Controls	Positive control not required for study type (OECD 412)	Not Rated	NA	NA	NA
	6. Randomized Allocation	Animals were "randomly selected for each exposure group".	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Vapor generation method was adequately reported.	High	1	1	1
	8. Consistency of Exposure Administration	Exposure methods were consistent between groups.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure duration consistent with cited guideline (OECD 412)	High	1	1	1

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	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.	High	1	1	1
	12. Exposure Route and Method	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.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	High	1	2	2

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were the same between groups. All animals acclimated to exposure chambers for 5 days before exposure.	High	1	1	1
	15. Number per Group	16/sex/group; 8/sex sacrificed at end of exposure, 8/sex sacrificed 2 weeks after exposure (minimum guideline: 5/sex/group per sacrifice)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	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	High	1	2	2
	17. Consistency of Outcome Assessment	Assessment identical across groups.	High	1	1	1
	18. Sampling Adequacy	Sampling consisted with cited guideline (OECD 412)	High	1	1	1
	19. Blinding of Assessors	Only non-subjective outcomes and initial histopathological evaluations performed; blinding not necessary.	Not Rated	NA	NA	NA

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	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)	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	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.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Lesion incidence compared with Fisher's exact test. Continuous data analyzed by t-test and ANOVA.	High	1	1	1

Study reference:	Mattie, D. R.,Bucher, T. W.,Carter, A. L.,Stoffregen, D. E.,Reboulet, J. E. (2012). Acute Inhalation Toxicity Study of 1, 4-Dioxane in Rats (Rattus norvegicus) GRA and I(20), 29. HERO ID: 3563367					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Quantitative reporting of lesions. Qualitative negative result reporting for hematology and clinical chemistry. Organ weights not reported. Likely no effect (no impact on outcome), so rated as medium.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**37**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2759**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

3. Subchronic and Chronic Toxicity Studies (Including Cancer)

3.1. 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 reference:	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. HERO ID: 196245					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name; no concern with different forms or mixtures.	High	1	2	2
	2. Test Substance Source	Test substance obtained from commercial source. and its purity established by IS and GC.	High	1	1	1
	3. Test Substance Purity	Test substance obtained from commercial source; purity >99.0% verified by IS and GC.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Control group received vehicle (deionized water); all groups were body-weight matched (stratified randomization).	High	1	2	2
	5. Positive Controls	Not indicated for study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	Group assignments by stratified randomization into body-weight matched groups.	High	1	1	1

Study reference:	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. HERO ID: 196245					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	High	1	1	1
	8. Consistency of Exposure Administration	Daily water intake calculated as difference between weight of water remaining in bottle 3-4 days after preparation divided by number of days.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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).	High	1	2	2
	10. Exposure Frequency and Duration	Frequency was not specified but is inferred to be 7 days per week; duration specified as 13 weeks.	High	1	1	1

Study reference:	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. HERO ID: 196245					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	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.	High	1	1	1
	12. Exposure Route and Method	Exposure route was reported and appropriate (drinking water).	High	1	1	1
Test Organism	13. Test Animal Characteristics	Test animal species, strain, age, and source were all reported and appropriate for subchronic toxicity evaluation.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	No differences between groups in animal husbandry conditions were reported. Animals were housed individually.	High	1	1	1
	15. Number per Group	Study used 10 animals/sex/group, which exceeds numbers recommended by OECD (5/sex/grp)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment was described in detail including organs/endpoints, methods, instrumentation, stains, and timing. Endpoints evaluated were sensitive for systemic toxicity.	High	1	2	2
	17. Consistency of Outcome Assessment	No inconsistencies in protocol execution were noted in the report.	High	1	1	1

Study reference:	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. HERO ID: 196245					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	All standard endpoints were evaluated in all animals of all exposure groups. ALtered hepatic foci evaluated in subsets of high exposure and control groups.	High	1	1	1
	19. Blinding of Assessors	There were no subjective outcomes evaluated.	Not Rated	NA	NA	NA
	20. Negative Control Response	Adequately reported.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Unacceptable	4	2	8
	22. Health Outcomes Unrelated to Exposure	Animal attrition was limited to two deaths (one rat and one mouse). No infections or other health outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were clearly described and appropriate for the data.	High	1	1	1
	24. Reporting of Data	Data for all groups on exposure-related findings were reported. Measures of variation and numbers of animals examined were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**35**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NA**	**Overall Score: Nearest *:**	**NA**
		Overall Quality Level:		**Medium**		

Study reference:	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. HERO ID: 196245					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Quality Comment:	The reviewer upgraded this study's overall quality rating, changing its status from unacceptable to acceptable. They noted: Although there was a dose-related decrease in water intake that exceeded 20% at the highest 2-3 exposure levels, data from the lower exposure groups may be useful. All other metrics were rated high. The study was initially assigned a rating of unacceptable (score = 4) with a calculated score of 1.2 (shown solely for transparency). No calculated score is identified for the current rating in the table above because the study was upgraded to medium.					

3.2. 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 reference:	**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. HERO ID: 195044**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Reagent grade 1,4-Dioxane (>99% pure); liquid	High	1	2	2
	2. Test Substance Source	Obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Batch number not provided, but identity and composition verified by laboratory using GC-MS.	High	1	1	1
	3. Test Substance Purity	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.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent control group exposed to clean air under same conditions as test groups.	High	1	2	2
	5. Positive Controls	Positive control group is not needed in standard 13-wk inhalation study (see OECD guideline 413)	Not Rated	NA	NA	NA
	6. Randomized Allocation	stratified randomization into 8 body-weight-matched groups, each comprised of 10 rats/sex	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Detailed description of vapor generation; chamber concentrations of 1,4-dioxane monitored every 15 minutes during exposure;	High	1	1	1

Study reference:	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. HERO ID: 195044					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	Exposure conditions identical between groups (except exposure levels). All animals in an exposure group were exposed simultaneously (exposure chamber held 20 individual cages).	High	1	1	1
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	Consisted with cited OECD guideline 413 (6 h/d, 5 d/wk, 13 wk)	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	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).	Medium	2	1	2

Study reference:	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. HERO ID: 195044					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	Detailed description of vapor generation and whole-body exposure conditions (1060 L exposure chambers, housed 20 individual cages).	High	1	1	1
Test Organism	13. Test Animal Characteristics	Six-week-old F344/DuCrj rats of both sexes (obtained at 4-weeks of age)	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Housing conditions described adequately; same conditions in control and exposure groups.	High	1	1	1
	15. Number per Group	10/sex/group, as per cited OECD guideline 413	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	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	High	1	2	2

Study reference:	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. HERO ID: 195044					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	17. Consistency of Outcome Assessment	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.	High	1	1	1
	18. Sampling Adequacy	Sampling consistent with cited OECD guideline 413.	High	1	1	1
	19. Blinding of Assessors	Blinding status of assessors was not reported, Evaluated endpoints included non-subjective metrics and initial histopathology review, so blinding was not needed.	Not Rated	NA	NA	NA
	20. Negative Control Response	Control results were reported, and within expected biological variation.	High	1	1	1

Study reference:	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. HERO ID: 195044					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Mortality was limited to the high-exposure group, and was attributed to exposure-related effects (renal failure)	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	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.	High	1	1	1

Study reference:	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. HERO ID: 195044					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	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).	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**34**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1724**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

3.3. 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 reference:	**Kociba, R. J.,McCollister, S. B.,Park, C.,Torkelson, T. R.,Gehring, P. J. (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286. HERO ID: 62929**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Clearly identifies substance as 1,4-dioxane	High	1	2	2
	2. Test Substance Source	Compound obtained from The Dow Chemical Co. (batch no. not reported).	Medium	2	1	2
	3. Test Substance Purity	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%.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Untreated controls were given regular drinking water.	High	1	2	2
	5. Positive Controls	Positive control not warranted by study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The study did not report how animals were allocated to study groups	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	High	1	1	1

Study reference:	Kociba, R. J.,McCollister, S. B.,Park, C.,Torkelson, T. R.,Gehring, P. J. (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286. HERO ID: 62929					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	Drinking water was available ad libitum to all exposure groups.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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.	High	1	2	2
	10. Exposure Frequency and Duration	2 yr study; drinking water available ad libitum	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	3 dose groups - low dose did not induce toxic effects or tumors; mid-dose induced some toxic effects, high-dose induced tumors.	High	1	1	1
	12. Exposure Route and Method	drinking water administration	High	1	1	1
Test Organism	13. Test Animal Characteristics	6-8 wk old Sherman rats; male and female	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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.	Medium	2	1	2

Study reference:	Kociba, R. J.,McCollister, S. B.,Park, C.,Torkelson, T. R.,Gehring, P. J. (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286. HERO ID: 62929					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	60/sex/group	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Cancer: complete histological analysis, sufficient duration of study Renal: OW, histopathology Hepatic: OW, histopathology Hematology, Bd wt, mortality - adequately assessed	High	1	2	2
	17. Consistency of Outcome Assessment	The same protocols were used for control and exposure groups.	High	1	1	1
	18. Sampling Adequacy	Adequate numbers were used in all groups. Effective number of animals for tumor analysis was calculated.	High	1	1	1
	19. Blinding of Assessors	All evaluations were non-subjective or initial histopathological evaluations.	Not Rated	NA	NA	NA
	20. Negative Control Response	Control results reported, no noted deviations from expectation.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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).	High	1	2	2

Study reference:	Kociba, R. J.,McCollister, S. B.,Park, C.,Torkelson, T. R.,Gehring, P. J. (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats Toxicology and Applied Pharmacology, 30(2), 275-286. HERO ID: 62929					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	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.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	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.	High	1	1	1
	24. Reporting of Data	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	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**35**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2069**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

3.4. 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 reference:	**Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was clearly identified by name.	High	1	2	2
	2. Test Substance Source	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.	Medium	2	1	2
	3. Test Substance Purity	The test substance purity was reportedly 99.9%; therefore, any effects observed are likely due to the nominal test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	The study authors reported using an appropriate concurrent negative control group (rats exposed to filtered air only).	High	1	2	2
	5. Positive Controls	Positive controls not indicated by study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The study authors did not indicate how animals were allocated to study groups,	Low	3	1	3

Study reference:	Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	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.	Medium	2	1	2
	9. Reporting of Doses/ Concentrations	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).	High	1	2	2
	10. Exposure Frequency and Duration	Exposure frequency and duration were suited to the study type and outcome of interest.	High	1	1	1

Study reference:	Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	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.	Unacceptable	4	1	4
	12. Exposure Route and Method	Rats were exposed to the test substance under dynamic exposure conditions.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	Medium	2	2	4

Study reference:	Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were not reported in sufficient detail to determine if conditions were the same/adequate between control and exposed groups.	Low	3	1	3
	15. Number per Group	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.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome methodology addressed the intended outcomes of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes appear to have been assessed consistently across groups (same time after initial exposure) and using the same protocols.	High	1	1	1
	18. Sampling Adequacy	Endpoints (including hematology and clinical chemistry, gross and microscopic pathology) were evaluated in all surviving animals.	High	1	1	1
	19. Blinding of Assessors	Blinding not required for initial histopathology examinations (other endpoints evaluated were not subjective).	Not Rated	NA	NA	NA

Study reference:	Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	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."	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes not related to exposure were not reported because there were not any significant differences among groups.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were described (in minimal detail) and appear to be appropriate.	High	1	1	1
	24. Reporting of Data	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).	Medium	2	2	4

Study reference:	Torkelson, T. R.,Leong, B. K. J.,Kociba, R. J.,Richter, W. A.,Gehring, P. J. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats Toxicology and Applied Pharmacology, 30(2), 287-298. HERO ID: 94807					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**45**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.5517**	**Overall Score (Rounded):**	**1.6[1]**
		Overall Quality Level:		**Unacceptable[1]**		

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

3.5. Animal toxicity evaluation results of Argus et al 1965 for a cancer bioassay-liver, kidney, blood study on cancer outcomes

Study reference:	**Argus, M. F., Arcos, J. C.,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. HERO ID: 17009**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name and chemical formula and structure	High	1	2	2
	2. Test Substance Source	Eastman organic chemical number was reported	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	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.	Low	3	2	6
	5. Positive Controls	The metric is not applicable.	Not Rated	NA	NA	NA
	6. Randomized Allocation	How animals were allocated was not reported.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	Treated animals had access to drinking water continuously	High	1	1	1

Study reference:	Argus, M. F., Arcos, J. C.,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. HERO ID: 17009					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/ Concentrations	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.	Medium	2	2	4
	10. Exposure Frequency and Duration	Data found in Table 1.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Only one treatment dose was used	Not Rated	NA	NA	NA
	12. Exposure Route and Method	Exposure through drinking water was acceptable as 1,2-dioxane can leach into and remain in water	High	1	1	1
Test Organism	13. Test Animal Characteristics	Animal source, species, strain, sex, life-stage, and body weight range were reported. Specific age and health status was not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Limited husbandry conditions were reported, but appear to be similar among the groups.	Medium	2	1	2
	15. Number per Group	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.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Limited details regarding the complete necropsy and histological investigation were reported.	Medium	2	2	4

Study reference:	Argus, M. F., Arcos, J. C.,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. HERO ID: 17009					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	17. Consistency of Outcome Assessment	Based on the study report, it is inferred that outcome assessment was consistent.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	1	1
	19. Blinding of Assessors	This metric is not applicable.	Not Rated	NA	NA	NA
	20. Negative Control Response	Biological responses were adequate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	The lack of reported of initial body weight and specific water intake is not likely to have a substantial impact on results.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure were not reported.	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was not conducted, but some data were provided which could be used to do an independent analysis (incidence of rats with tumors)	Low	3	1	3
	24. Reporting of Data	Tabular data for tumor outcomes was reported, all other data were described in the text and incidence and severity data were not reported.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**28**	**54**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.9286**	**Overall Score: Nearest *:**	**1.9**
		Overall Quality Level:		**Medium**		

3.6. Animal toxicity evaluation results of Argus et al 1973 for a carcinogenicity-liver (dose response), electron microscopy study on cancer outcomes

Study reference:	**Argus, M. F.,Sohal, R. S.,Bryant, G. M.,Hoch-Ligeti, C.,Arcos, J. C. (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity European Journal of Cancer, 9(4), 237-243. HERO ID: 62912**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by name and source same as Argus et al., 1965 , which limits uncertainties	Medium	2	2	4
	2. Test Substance Source	Source reported but no additional details were reported	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	There were no apparent differences in the concurrent control group.	High	1	2	2
	5. Positive Controls	This metric was not applicable.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The study did not report how animals were allocated to study groups.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Solutions were prepared fresh daily in drinking water.	High	1	1	1
	8. Consistency of Exposure Administration	There were no apparent inconsistencies in exposure administration.	High	1	1	1
	9. Reporting of Doses/ Concentrations	The doses were reported along with average fluid consumption	High	1	2	2
	10. Exposure Frequency and Duration	Duration was provided	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and dose spacing were appropriate	High	1	1	1
	12. Exposure Route and Method	The route and method were appropriate.	High	1	1	1

Study reference:	Argus, M. F.,Sohal, R. S.,Bryant, G. M.,Hoch-Ligeti, C.,Arcos, J. C. (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity European Journal of Cancer, 9(4), 237-243. HERO ID: 62912					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The species, strain, sex, age, initial body weight range, and source were reported. The health status of the animals was not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were not sufficiently reported to evaluate if adequate.	Low	3	1	3
	15. Number per Group	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.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Limited details in outcome assessment methodology was provided.	Medium	2	2	4
	17. Consistency of Outcome Assessment	It is inferred that outcome assessment was consistent.	High	1	1	1
	18. Sampling Adequacy	All animals were assessed.	High	1	1	1
	19. Blinding of Assessors	This metric is not applicable.	Not Rated	NA	NA	NA
	20. Negative Control Response	The biological responses of the control animals in the dose response study were not reported.	Unacceptable	4	1	4
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No differences were reported.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Details were not reported	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were not reported	Low	3	1	3

Study reference:	Argus, M. F.,Sohal, R. S.,Bryant, G. M.,Hoch-Ligeti, C.,Arcos, J. C. (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity European Journal of Cancer, 9(4), 237-243. HERO ID: 62912					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Data were described in the text, and descriptive tumor characteristics were not distinguished among groups. Effective tumor doses were reported	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**54**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NA**	**Overall Score: Nearest *:**	**NA**
		Overall Quality Level:		**Low**		
Study Quality Comment:	**The reviewer upgraded this study's overall quality rating, changing its status from unacceptable to acceptable. They noted: The study would be upgraded because a description of the tumors observed was provided which is informative. Also, effective tumor doses were provided. The study was initially assigned a rating of unacceptable (score = 4) with a calculated score of 1.9 (shown solely for transparency). No calculated score is identified for the current rating in the table above because the study was upgraded to low.**					

3.7. 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 reference:	**JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water). HERO ID: 196240**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by name, structure, and CASRN	High	1	2	2
	2. Test Substance Source	Source was reported but no additional information.	Medium	2	1	2
	3. Test Substance Purity	Purity such that effects likely due to test substance	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Appropriate negative control group was included	High	1	2	2
	5. Positive Controls	Not applicable for this study	Not Rated	NA	NA	NA
	6. Randomized Allocation	Allocation of animals was not reported	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance was administered in the drinking water, but additional details were not reported..	Low	3	1	3
	8. Consistency of Exposure Administration	Exposures were consistent	High	1	1	1
	9. Reporting of Doses/ Concentrations	See footnote at end of page.[1]	High	1	2	2
	10. Exposure Frequency and Duration	See footnote at end of page.[1]	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	See footnote at end of page.[1]	High	1	1	1
	12. Exposure Route and Method	See footnote at end of page.[1]	High	1	1	1

[1] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

Study reference:	JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water). HERO ID: 196240					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The source, species, strain, sex, and age were reported. Starting body weight and health status were not reported	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	All husbandry conditions were reported.	High	1	1	1
	15. Number per Group	See footnote at end of page.[1]	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome methodology was appropriate and sensitive	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently	High	1	1	1
	18. Sampling Adequacy	Sampling was appropriate	High	1	1	1
	19. Blinding of Assessors	Not applicable for this study	Not Rated	NA	NA	NA
	20. Negative Control Response	See footnote at end of page.[1]	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	See footnote at end of page.[1]	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	There were no differences among groups unrelated to exposure	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical analyses were reported and appropriate	High	1	1	1
	24. Reporting of Data	Outcomes were reported.	High	1	2	2

[1] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

Study reference:	JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water). HERO ID: 196240					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**36**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2414**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

3.8. Animal toxicity evaluation results of Kano et al 2009 for a 2-year cancer bioassay study on cancer outcomes

Study reference:	**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. HERO ID: 594539**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by CASRN and each lot analyzed by IR and GC.	High	1	2	2
	2. Test Substance Source	Obtained from manufacturer.	High	1	1	1
	3. Test Substance Purity	>99% pure; confirmed by GC	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Adequately reported	High	1	2	2
	5. Positive Controls	Not indicated for study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	Stratified randomization; matched by body weight	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Adequately reported; prepared twice per week and stable at 4 days post-preparation.	High	1	1	1
	8. Consistency of Exposure Administration	Drinking water available to all animals ad libitum	High	1	1	1
	9. Reporting of Doses/ Concentrations	Data provided on water consumption; no difference across groups.	High	1	2	2
	10. Exposure Frequency and Duration	Consistent with test guideline for study type.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Highest dose chosen so as not to exceed the MTD.	High	1	1	1
	12. Exposure Route and Method	Adequately reported. Consistent with test guideline for study type.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Adequately reported. Consistent with test guidelines for study type.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Adequately reported. Consistent with test guidelines for study type.	High	1	1	1

Study reference:	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. HERO ID: 594539					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	50/sex/group; consistent with test guidelines for study type.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Consistent with test guidelines for study type.	High	1	2	2
	17. Consistency of Outcome Assessment	No anomalies reported.	High	1	1	1
	18. Sampling Adequacy	Consistent with test guidelines for study type.	High	1	1	1
	19. Blinding of Assessors	Not addressed.	Low	3	1	3
	20. Negative Control Response	Adequately reported; no unusual results.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Body-weight matching; no difference in food/water consumption.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Attrition was related to exposure.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Appropriate methods chosen; adequately reported.	High	1	1	1
	24. Reporting of Data	Multiple data tables summarize all endpoints.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**32**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.0667**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

3.9. 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 reference:	**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. HERO ID: 193803**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified definitively.	High	1	2	2
	2. Test Substance Source	The source of the test substance was reported, including manufacturer, and its identity was verified by analytical methods.	High	1	1	1
	3. Test Substance Purity	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.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	The study authors reported using an appropriate concurrent negative control group.	High	1	2	2
	5. Positive Controls	Not applicable - Positive control group is not indicated by study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	The animals were divided by stratified randomization into body weight-matched groups.	High	1	1	1

Study reference:	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. HERO ID: 193803					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	High	1	1	1
	8. Consistency of Exposure Administration	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.	Medium	2	1	2
	9. Reporting of Doses/ Concentrations	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).	High	1	2	2
	10. Exposure Frequency and Duration	The exposure frequency and duration of exposure were reported and were appropriate for this type of study.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and concentration spacing were justified and adequate for the purpose of this study.	High	1	1	1

Study reference:	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. HERO ID: 193803					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	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).	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	All husbandry conditions were reported and were adequate and consistent among the groups and controls.	High	1	1	1
	15. Number per Group	The number of animals per study group was reported and appropriate for the study type.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	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.	Low	3	1	3

Study reference:	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. HERO ID: 193803					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	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).	High	1	1	1
	19. Blinding of Assessors	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.	Not Rated	NA	NA	NA
	20. Negative Control Response	The negative control response was adequate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	No indications of attrition or health outcomes unrelated to exposure.	High	1	1	1

Study reference:	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. HERO ID: 193803					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	The statistical methods were clearly described and appropriate for the data set.	High	1	1	1
	24. Reporting of Data	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.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**41**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.4138**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

3.10. Animal toxicity evaluation results of NCI et al 1978 for a cancer bioassay- male rats study on cancer outcomes

Study reference:	**NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified by name and CASRN.	High	1	2	2
	2. Test Substance Source	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).	High	1	1	1
	3. Test Substance Purity	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.	High	1	1	1

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Design	4. Negative and Vehicle Controls	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.	Unacceptable	4	2	8
	5. Positive Controls	Positive control group not indicated by study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	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).	Low	3	1	3

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	Dosed water or tap water was available ad libitum.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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."	Low	3	2	6

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Exposure Frequency and Duration	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.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	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.	Low	3	1	3
	12. Exposure Route and Method	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.	Medium	2	1	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	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).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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).	High	1	1	1

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	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).	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	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.	Medium	2	2	4
	17. Consistency of Outcome Assessment	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.	Medium	2	1	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	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.	Medium	2	1	2
	19. Blinding of Assessors	Blinding not reported, but is not required for initial histopathology review.	Not Rated	NA	NA	NA
	20. Negative Control Response	The biological responses of the negative control groups were adequate (showing no or low incidences of lesions).	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	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.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	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.	Medium	2	1	2
	24. Reporting of Data	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.	High	1	2	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		Sum of scores:			29	55
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		1.8966	Overall Score (Rounded):	1.9[1]
		Overall Quality Level:		Unacceptable[1]		

Footnote:

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

3.11. 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 reference:	**NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified by name and CASRN.	High	1	2	2
	2. Test Substance Source	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).	High	1	1	1
	3. Test Substance Purity	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.	High	2	1	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Design	4. Negative and Vehicle Controls	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.	Low	3	2	6
	5. Positive Controls	Positive control group not indicated by study type.	Not Rated	NA	NA	NA
	6. Randomized Allocation	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).	Low	3	1	3

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	Medium	2	1	2
	8. Consistency of Exposure Administration	Dosed water or tap water was available ad libitum.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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."	Low	3	2	6

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Exposure Frequency and Duration	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.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	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.	Low	3	1	3

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	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.	High	2	1	2
Test Organism	13. Test Animal Characteristics	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).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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).	High	2	1	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	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).	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	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.	Medium	2	2	4
	17. Consistency of Outcome Assessment	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.	Medium	2	1	2

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	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.	Medium	2	1	2
	19. Blinding of Assessors	Blinding not reported, but is not required for initial histopathology review.	Not Rated	NA	NA	NA
	20. Negative Control Response	The biological responses of the negative control groups were adequate (showing no or low incidences of lesions).	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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.	Low	3	2	6

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	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.	High	2	1	2
Data Presentation and Analysis	23. Statistical Methods	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.	Medium	2	1	2
	24. Reporting of Data	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.	High	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**58**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NA**	**Overall Score: Nearest *:**	**NA**
		Overall Quality Level:		**Low**		

Study reference:	NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. HERO ID: 62935					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: The study has some numerous limitations. Some of these data might be usable (if dosing permutations are permitted); namely carcinogenicity data for female rats and male and female mice. Note: The original calculated score for this study was 1.7. This value is not presented above because the final rating was changed based on professional judgement.**					

4. In Vivo and In Vitro Genotoxicity Studies

These studies include acute and short-term exposure duration studies. Note that one in vivo study includes hepatic and body weight outcomes and another includes gene expression data.

4.1. 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 reference:	**Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	1,4-dioxane	High	1	2	2
	2. Test Substance Source	Baker Chemical Company; no batch number, but purity was analyzed by study laboratory	Medium	2	1	2
	3. Test Substance Purity	>99%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent vehicle (saline) control was used	High	1	2	2
	5. Positive Controls	No positive control; in vivo genotoxicity study design indicates one should have been used (DMN was used in the repeat dose study only)	Unacceptable	4	1	4
	6. Randomized Allocation	Animals were computer randomized into treatment groups in all experiments	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Storage details not reported. Mixed with saline for gavage administration.	High	1	1	1
	8. Consistency of Exposure Administration	Exposure conditions consistent between groups.	High	1	1	1
	9. Reporting of Doses/ Concentrations	Replicate 1: 0, 100, or 1000 mg/kg Replicate 2: 0, 10, 100, or 1000 mg/kg	High	1	2	2
	10. Exposure Frequency and Duration	Once, sacrificed after 7 d	High	1	1	1

Study reference:	Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	2-3 doses plus negative control (two replicates)	High	1	1	1
	12. Exposure Route and Method	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.	Medium	2	1	2
Test Organism	13. Test Animal Characteristics	Male SD rats (Spartan Research). Based on weight (180-260g), they were adult animals.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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.	Medium	2	1	2
	15. Number per Group	4/group	High	1	1	1

Study reference:	Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Genotox, organ weight, and histology of liver (cancer target organ); body weight and food consumption also monitored.	High	1	2	2
	17. Consistency of Outcome Assessment	Consistent evaluation across study groups	High	1	1	1
	18. Sampling Adequacy	4/group	High	1	1	1
	19. Blinding of Assessors	Only non-subjective and initial histological evaluations; blinding not required.	Not Rated	NA	NA	NA
	20. Negative Control Response	negative control response was reported; no deviations from normal were reported.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	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).	Unacceptable	4	2	8
	22. Health Outcomes Unrelated to Exposure	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.	Low	3	1	3

Study reference:	Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	Continuous data were compared by Dunnett's t-test. No statistical analysis of histopathological findings. Histological findings only reported qualitatively.	Medium	2	1	2
	24. Reporting of Data	DNA synthesis, liver weight, and BWG reported quantitatively with statistics. Histopathological findings reported qualitatively (present or absent at dose).	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			30	47
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.5667**	**Overall Score (Rounded):**	**1.6[1]**
		Overall Quality Level:		**Unacceptable[1]**		

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

4.2. Animal toxicity evaluation results of Itoh 2019 - in vivo genotoxicity assay - micronucleus test

Study reference:	**S. Itoh (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. HERO ID: 5072318**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	1,4-dioxane (CAS No. 123-91-1)	High	1	2	2
	2. Test Substance Source	Wako Pure Chemical Industries, Ltd. (Osaka, Japan)	High	1	1	1
	3. Test Substance Purity	The purity and/or grade were not reported	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent vehicle control	High	1	2	2
	5. Positive Controls	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]	High	1	1	1
	6. Randomized Allocation	The study did not report how animals were allocated to study groups	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	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.	High	1	1	1
	8. Consistency of Exposure Administration	Exposure administration was consistent across exposure groups for each experiment.	High	1	1	1
	9. Reporting of Doses/ Concentrations	0, 1000, 2000, or 3000 mg/kg	High	1	2	2

Study reference:	S. Itoh (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. HERO ID: 5072318					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Exposure Frequency and Duration	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	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	0, 1000, 2000, or 3000 mg/kg based on previous reports	High	1	1	1
	12. Exposure Route and Method	Gavage at dose volume of 10 mL/kg	High	1	1	1
Test Organism	13. Test Animal Characteristics	Male F344/DuCrlCrlj rats, 4- to 8-wks of age; Charles River Laboratories Japan, Inc.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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	High	1	1	1
	15. Number per Group	4-5/group per test	High	1	1	1

Study reference:	S. Itoh (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. HERO ID: 5072318					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	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)	High	1	2	2
	17. Consistency of Outcome Assessment	See footnote at end of page.[3]	High	1	1	1
	18. Sampling Adequacy	See footnote at end of page.[1]	High	1	1	1
	19. Blinding of Assessors	All quantitative measures	Not Rated	NA	NA	NA
	20. Negative Control Response	See footnote at end of page.[1]	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Initial BW not reported; not likely to have substantial impact	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	See footnote at end of page.[1]	High	1	1	1

[3] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

Study reference:	S. Itoh (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. HERO ID: 5072318					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	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	High	1	1	1
	24. Reporting of Data	Graphical reporting of all genotox data; quantitative reporting for relative liver weight data	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**36**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

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

Study reference:	**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. HERO ID: 194385**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance was identified definitively.	High	1	2	2
	2. Test Substance Source	The source of the test substance was reported (Tokyo Chem Industry Co). A batch/lot number was not reported.	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	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).	Low	3	2	6
	5. Positive Controls		Not Rated	NA	NA	NA
	6. Randomized Allocation	The study authors did not report how animals were allocated to study groups.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	The test substance was dissolved or suspended in corn oil; however, no other details were provided on test substance preparation or storage methods.	Low	3	1	3
	8. Consistency of Exposure Administration	Details of exposure were reported and there was no indication to suggest that the exposures differed among the groups.	High	1	1	1

Study reference:	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. HERO ID: 194385					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/ Concentrations	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.	Medium	2	2	4
	10. Exposure Frequency and Duration	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).	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	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).	High	1	1	1
	12. Exposure Route and Method	The exposure route and method were reported and were considered appropriate for the purpose of the study.	High	1	1	1
Test Organism	13. Test Animal Characteristics	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.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Most husbandry conditions (temperature and light) were reported and were similar for all groups. Humidity was not reported.	Medium	2	1	2

Study reference:	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. HERO ID: 194385					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	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.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	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.	Medium	2	2	4
	17. Consistency of Outcome Assessment	The outcome assessment methodology appeared to be consistent among the groups.	High	1	1	1
	18. Sampling Adequacy	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).	Medium	2	1	2
	19. Blinding of Assessors	No subjective outcomes were evaluated in this study.	Not Rated	NA	NA	NA
	20. Negative Control Response	Biological responses of the negative control group were adequate.	High	1	1	1

Study reference:	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. HERO ID: 194385					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No confounding variables in test design were reported; however, initial body weight and food/water intake were not reported.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	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.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were reported and were appropriate for the data sets.	High	1	1	1
	24. Reporting of Data	Data for exposure-related findings were presented (RDS incidence and cell viability, only mechanistic outcomes were reported).	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**52**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.7931**	**Overall Score: Nearest *:**	**1.8**
		Overall Quality Level:		**Medium**		

4.4 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 reference:	**Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Reported only as "1,4-dioxane".	Low	3	2	6
	2. Test Substance Source	Baker Chemical Company; no batch number, but purity was analyzed by study laboratory	Medium	2	1	2
	3. Test Substance Purity	>99%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent vehicle (saline) control was used	High	1	2	2
	5. Positive Controls	Known genotoxic agent dimethylnitrosamine (DMN) was used as a positive control	High	1	1	1
	6. Randomized Allocation	Animals were computer randomized into treatment groups in all experiments	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Storage details not reported. Mixed with drinking water. No details on frequency of drinking water preparation.	Low	3	1	3
	8. Consistency of Exposure Administration	Exposure conditions consistent between groups.	High	1	1	1
	9. Reporting of Doses/ Concentrations	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.	Low	3	2	6
	10. Exposure Frequency and Duration	11 weeks, 7d/wk	High	1	1	1

Study reference:	Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	2 doses plus negative and positive control	High	1	1	1
	12. Exposure Route and Method	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.	Medium	2	1	2
Test Organism	13. Test Animal Characteristics	Male SD rats (Spartan Research). Based on weight (180-260g), they were adult animals.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	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.	Medium	2	1	2
	15. Number per Group	5-6/group	High	1	1	1

Study reference:	Dow Chemical, Co (1989). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. HERO ID: 4158030					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Genotox, organ weight, and histology of liver (cancer target organ); body weight and food consumption also monitored.	High	1	2	2
	17. Consistency of Outcome Assessment	Consistent evaluation across study groups	High	1	1	1
	18. Sampling Adequacy	5-6/group	High	1	1	1
	19. Blinding of Assessors	Only non-subjective and initial histological evaluations; blinding not required.	Not Rated	NA	NA	NA
	20. Negative Control Response	negative control response was reported; no deviations from normal were reported.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Initial BW 180-260g (not reported per group). Body weight gains similar between groups during study.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	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	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Continuous data were compared by Dunnett's t-test. No statistical analysis of histopathological findings. Histological findings only reported qualitatively.	Medium	2	1	2