



United States
Environmental Protection Agency

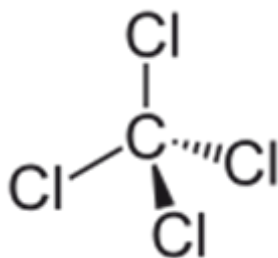
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Carbon Tetrachloride

Systematic Review Supplemental File:

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

CASRN: 56-23-5



October 2020

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1 Acute (<24 hr)

Table 1: Animal toxicity evaluation results of Roudabush et al., 1965 for an acute dermal toxicity and dermal irritation studies study on acute toxic-ity/poisoning and irritation outcomes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|--|
| Study Citation: Roudabush, RL; Terhaar, CJ; Fassett, DW; Dziuba, SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs Toxicology and Applied Pharmacology, 7(4), 559-565 | | | | | |
| Data Type: acute dermal toxicity and dermal irritation studies | | | | | |
| HERO ID: 79743 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was clearly identified. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The manufacturer was reported. The batch lot number for materials was not reported; however, this omission is unlikely to have a substantial impact on result. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Purity or chemical grade was not reported.; however given other information, purity was not expected to be of concern. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The use of controls were not discussed in the methodology sections of the report; however, the results table of the dermal irritation tests reported results for distilled water. The standard test guidelines (e.g., OECD) do not require negative controls for acute toxicity studies. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Not applicable for this study type. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups for either the acute toxicity nor the irritation studies. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test materials were noted to be undiluted. Storage conditions were not reported; however, omission of these details are unlikely to have a substantial impact on results. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was reported for both studies and were administered consistently across study groups for both species. |
| Metric 9: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | Study report does not specify the administered dermal doses for the acute toxicity study or the irritation study for either species. The report only states "a minimum of 3 dosages was employed" for the acute dermal toxicity test.. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The report notes that the procedure followed the protocols described in the Regulations (21 CFR 191.10), which includes details on exposure duration for both the acute dermal toxicity test (24 hours) and the primary irritation test (24 hours and 72 hours). |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Unacceptable | × 1 | 4 | The number of exposure groups was stated as "a minimum of 3 dosages" for the acute dermal toxicity study; the actual number of dose groups and spacing is not reported. The dosing of the irritation study is also not reported. |
| Continued on next page ... | | | | | |

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| Study Citation: | Roudabush, RL; Terhaar, CJ; Fassett, DW; Dziuba, SP (1965). Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs Toxicology and Applied Pharmacology, 7(4), 559-565 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | acute dermal toxicity and dermal irritation studies | | | | | |
| HERO ID: | 79743 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The route and method of exposure was reported and appropriate for the study types | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | There are deficiencies in the reporting of the test animal characteristics. The strain of guinea pigs and rabbits, sex used for each study, and the starting body weight ranges were reported. There is some uncertainty in the source of white rabbits (reported to be from a "local supplier"). These uncertainties are unlikely to have a substantial impact on results. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not reported. to evaluate if husbandry was adequate and if differences occurred between control and exposed groups.. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The reported number of animals per study group for the acute toxicity test was unclear (reported to be "usually" 4 animals/dose group). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcome(s) of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | There was incomplete reporting of minor details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results. | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Details on outcome assessments sampling were not reported, but is likely that all tested animals were sampled. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | The study types do not require blinding of assessors. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological response of the negative control group (distilled water) was adequate for the dermal irritation study. There was no reported control used in the acute toxicity test. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | There were no reporting for any possible differences among the study groups that could influence the outcome assessment.; however, the lack of reporting is not likely to have a significant impact on results. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data for outcomes unrelated to exposure for each study group were not reported, but the lack of reporting is unlikely to influence the study results. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| Continued on next page ... | | | | | | |

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

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

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 2: **Animal toxicity evaluation results of Hayes et al., 1986 for an acute oral lethality study on mortality outcomes**

| Study Citation: | Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice <i>Fundamental and Applied Toxicology</i> , 7(3), 454-463 | | | | |
|--|--|---------------------|------|-------|--|
| Data Type: | acute oral lethality test in mice | | | | |
| HERO ID: | 194400 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name |
| | Metric 2: Test Substance Source | Medium | × 1 | 2 | Test substance source and lot number reported, but certification/analytical verification of identity was not. |
| | Metric 3: Test Substance Purity | High | × 1 | 1 | Test substance reported to be HPLC grade and >99% pure. |
| Domain 2: Test Design | | | | | |
| | Metric 4: Negative and Vehicle Controls | Not Rated | NA | NA | Negative control not required for acute lethality study. |
| | Metric 5: Positive Controls | Not Rated | NA | NA | Positive controls not typical for this study type. |
| | Metric 6: Randomized Allocation | Medium | × 1 | 2 | Study reports randomizing the mice but is not clear regarding the allocation. |
| Domain 3: Exposure Characterization | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Low | × 1 | 3 | Study reports daily preparation of solution, but does not report storage. |
| | Metric 8: Consistency of Exposure Administration | Not Rated | NA | NA | Only one exposure group tested |
| | Metric 9: Reporting of Doses/Concentrations | Medium | × 2 | 4 | Dose reported in mg/kg bw; body weight not reported. |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | |
| | Metric 11: Number of Exposure Groups and Dose Spacing | Unacceptable | × 1 | 4 | Single exposure group is not sufficient to determine LD50 |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | |
| Domain 4: Test Organism | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were described and appropriate. |
| | Metric 15: Number per Group | High | × 1 | 1 | 10/sex were tested; this is more than adequate for acute lethality |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Unacceptable | × 2 | 8 | Duration of post-exposure observation was not reported. |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Only one group tested. |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Mortality assessed in all exposed animals. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Mortality is not subjective |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | No negative control was used |
| Domain 6: Confounding / Variable Control | | | | | |

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

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|---|
| Study Citation: Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351 | | | | | |
| Data Type: acute percutaneous toxicity in guinea pig | | | | | |
| HERO ID: 61688 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | Medium | × 2 | 4 | The test substances were identified; however, the test substances were lacking characterization details; unlikely to have a substantial impact on results. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The source of the test substances were identified; did not include batch/lot numbers; unlikely to have a substantial impact on results. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | purity or grade of test substances were not reported; possible impurities were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Distilled water was used as a concurrent control |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not rated/applicable; positive control was not indicated by study type |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | There were no details of test substance preparation and/or storage conditions reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Single application to skin depot (31 cm ²) and covered CK: Not 31 cm ² . The solvents was administered to a skin depot area 3.1 cm ² |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | applied concentrations were reported in ml; mean body weight was reported to estimate an administered dose. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | single application, covered, and observed for 35 d |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | The number of exposure groups for CCl ₄ = 2 and TCE=1; number of exposure groups and spacing were not justified by the author; Doses were considered adequate to address the purpose of the study for changes in body weight for both CCl ₄ and TCE; however for TCE, it is unclear if the exposure level was adequate to show results relevant to mortality as there were no effects at the single concentration tested. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substances |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source, strain, or sex of the test guinea pigs were not reported. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate |
| | Metric 15: Number per Group | High | × 1 | 1 | 20 animals per series |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest; mortality was monitored and body weight was recorded |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Mortality observations and weight measurements were made for all animals daily except weekends |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | this metric is not rated/applicable because no subjective outcomes were assessed. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control group(s) were adequate |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial body weights were reported; there was no reporting of food/water intake; unlikely to have a significant impact on results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on attrition and/or health outcomes unrelated to exposure for each study group were not reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Low | × 1 | 3 | Noted that an analysis of variance was applied in the statistical calculations, though statistical tests were not specified. P-values (unspecified significance test) were reported for body weight changes. No statistical significance values were reported for mortality |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Incidence of mortality was reported for both CCl4 and TCE. Body weight changes was reported for TCE, but not CCl4 |
| Overall Quality Determination [‡] | | Medium | | 1.9 | |
| Extracted | | Yes | | | |

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | acute inhalation toxicity in rats | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name and contaminants identified. | |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants. | |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Negative controls not required for acute lethality test | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for acute lethality test | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Study did not describe method of animal allocation | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal. | |
| Metric 8: | Consistency of Exposure Administration | Low | × 1 | 3 | Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration. | |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method. | |
| Metric 10: | Exposure Frequency and Duration | Medium | × 1 | 2 | Exposure durations ranged between 0.1 and 12 hours. Acute inhalation lethality tests are typically 4 hours in duration. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 6 exposure groups ranging more than 6-fold (high to low) were used, but the durations of exposure varied by exposure. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Dynamic whole body chamber was used for vapor that may condense. | |
| Domain 4: Test Organism | | | | | | |
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| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|--|--|---------------------------|------|-------|---|--|
| Data Type: | acute inhalation toxicity in rats | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Low | × 2 | 6 | Test animal species, strain, and source (in-house colony) were reported. Study reports using both sexes but does not indicate which sex was used for each exposure level and duration. Initial health status, age, and body weight were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions other than the exposure chamber were not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | Between 5 and 20 animals were used for each combination of concentration and duration. This is more than required for an acute lethality study | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology and outcomes assessed were typical for acute lethality study. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | Study reports observing survivors for 2-3 weeks or until full recovery was established. This could lead to inconsistencies in mortality assessment if there are late deaths. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Mortality is not subjective outcome. | |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | Negative controls not required for acute lethality study. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food/water intake, and respiratory rate were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was not conducted, and an LC50 was not identified. Mortality data enabling independent statistical analysis were reported. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Mortality data are reported, but without time to death and not by sex. | |
| Overall Quality Determination [‡] | | Medium → Low [§] | | | 2.2 | |
| Extracted | | Yes | | | | |
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Study Citation: Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
 Data Type: acute inhalation toxicity in rats
 HERO ID: 62373

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "Varying numbers of animals were exposed to different concentrations for different durations and with varying postexposure observation times."

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

| Study Citation: | Kronevi, T; Wahlberg, J; Holmberg, B (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs Environmental Research, 19(1,1), 56-69 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | acute dermal | | | | | |
| HERO ID: | 3684159 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as carbon tetrachloride (p.a.). | |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Obtained from E. Merck, Darmstadt, Germany. No batch/lot number. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Not specified, but reported "p.a.", which indicates analytical grade | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | No control animals were used. Study authors note that skin morphology in exposed area was compared to skin morphology from unexposed area in the same animal. No comparator for liver or kidney histology. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Study authors did not report animal allocation methods. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Not Rated | NA | NA | Pure solvent was applied, so no preparation was required. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | All animals similarly exposed | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | 1 mL of pure solvent applied within a glass ring with an inside diameter of 20 mm (area 3.1 cm ²). At a density of 1.59 g/cm ³ = 1.59 g/mL, the administered dose was 1.59 g. Glass ring was covered with glass (occluded conditions). | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure was for 15 minutes, 1 hr, 4 hr, or 16 hr | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Only one dose group (pure solvent), but for 4 durations. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Dermal exposure using a covered glass ring to prevent volatilization or exposure via inhalation or oral routes. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | Albino guinea pigs weighting between 440 and 570 g. Source and sex of animals not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | No husbandry conditions were reported, but since this is an acute study this is not likely to have a major impact on study. | |
| Metric 15: | Number per Group | Unacceptable | × 1 | 4 | The number of animals per group were not explicitly reported. Overall number of animals was 20. There were 5 compounds tested, with each compound evaluated for 4 time-points. This implies that only one animal was used per compound per duration. | |

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| Study Citation: | Kronevi, T; Wahlberg, J; Holmberg, B (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs Environmental Research, 19(1,1), 56-69 | | | | |
|--|--|---------------------|------|-------|---|
| Data Type: | acute dermal | | | | |
| HERO ID: | 3684159 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Skin biopsy was performed on exposed area and neighboring un-exposed animal. Liver and kidney histology were assessed for evaluation of liver and kidney histology. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Only one animal per group and no controls, so sampling adequacy is N/A |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding is not required for initial histopathological review. |
| | Metric 20: Negative Control Response | Unacceptable | × 1 | 4 | Skin biopsy results from untreated skin were not reported. No control specimens for liver or kidney histology. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | Little concern with confounding in acute study design |
| | Metric 22: Health Outcomes Unrelated to Exposure | Not Rated | NA | NA | Attrition/infection N/A due to acute study design |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | No statistical methods. Only one animal per group, so data insufficient for statistical analysis. |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Results reported qualitatively. |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.0 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| Study Citation: Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
| Data Type: acute inhalation toxicity in rats | | | | | |
| HERO ID: 62373 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name and contaminants identified. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | Negative controls not required for acute lethality test, but neurotoxicity cannot be assessed without negative controls. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for acute lethality test |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Study did not describe method of animal allocation |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal. |
| Metric 8: | Consistency of Exposure Administration | Low | × 1 | 3 | Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration. |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure durations ranged between 0.1 and 12 hours. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 6 exposure groups ranging more than 6-fold (high to low) were used, but the durations of exposure varied by exposure. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Dynamic whole body chamber was used for vapor that may condense. |
| Domain 4: Test Organism | | | | | |

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| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | acute inhalation toxicity in rats | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Low | × 2 | 6 | Test animal species, strain, and source (in-house colony) were reported. Study reports using both sexes but does not indicate which sex was used for each exposure level and duration. Initial health status, age, and body weight were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions other than the exposure chamber were not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | Between 5 and 20 animals were used for each combination of concentration and duration. This should be adequate for acute toxicity | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | Frequency and timing of observation for clinical signs of neurotoxicity was not described. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | No information on consistency of clinical observations was provided. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Observations for clinical signs may be subjective and blinding was not reported. | |
| | Metric 19: Blinding of Assessors | Unacceptable | × 1 | 4 | | |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | Negative controls were not used. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food/water intake, and respiratory rate were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Statistical analysis was not conducted, and data enabling independent statistical analysis were not reported. | |
| | Metric 24: Reporting of Data | Unacceptable | × 2 | 8 | Incidences of clinical signs of neurotoxicity were not reported | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.6 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

2 Short-term (1-30 days)

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice <i>Fundamental and Applied Toxicology</i> , 7(3), 454-463 | | | | | |
| Data Type: 14 day oral toxicity test in mice | | | | | |
| HERO ID: 194400 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance source and lot number reported, but certification/analytical verification of identity was not. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance reported to be HPLC grade and >99% pure. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Both naive and sham-treated control groups were used. Sham-treated controls received vehicle. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for this study type. |
| Metric 6: | Randomized Allocation | Medium | × 1 | 2 | Study reports randomizing the mice but does not discuss the allocation to groups |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Study reports daily preparation of solution, but does not report storage. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Administration details are provided, including gavage volume and time of day of administration. No inconsistencies in exposures across groups were noted. |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | Dose reported in mg/kg bw; body weight not reported. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Animals gavaged daily for 14 days |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 3 nonzero doses ranging 4-fold were used. Effects were seen at all doses, so it is not clear that the lowest dose was low enough. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were described and appropriate. |
| Metric 15: | Number per Group | High | × 1 | 1 | 20/sex/dose were tested; this is more than adequate. |
| Domain 5: Outcome Assessment | | | | | |

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| Study Citation: | Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice <i>Fundamental and Applied Toxicology</i> , 7(3), 454-463 | | | | | |
|--|--|---------------------|------|------------|--|--|
| Data Type: | 14 day oral toxicity test in mice | | | | | |
| HERO ID: | 194400 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome assessment methodology was described in detail and appropriate. Histopathology was not evaluated, but organ weights, serum chemistry, and hematology were. Food and water intake were not reported. The only neurological and reproductive endpoints assessed were brain and testes weights, respectively. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | No inconsistencies in outcome assessment were noted by the authors apart from one gavage death in high dose females. | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Mortality, organ weights evaluated in all animals; hematology and serum chemistry evaluated in 5/sex/dose each. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | No subjective outcomes were evaluated | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Responses of both naive and vehicle controls were reported for all endpoints other than hematology. Responses appeared to be as expected and without excessive variability. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food and water intake were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | The study reports that 20 animals/sex/dose were tested, and that organ weights were evaluated in all animals; however, results are reported for only 10 animals/sex/dose. The study authors do not explain this discrepancy. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were reported and appropriate to the data. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Mortality, organ weights, and significant clinical chemistry findings were reported; body weight, hematology results, and non-significant clinical chemistry findings were not. The lack of body weight data is problematic for interpretation of relative organ weight changes. | |
| Overall Quality Determination[‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 8: **Animal toxicity evaluation results of Narotsky et al., 1997 study**

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

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

Data Type:

HERO ID: 194607

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|------------------------|
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | |
| Overall Quality Determination [‡] | | High | | 1.2 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 9: **Animal toxicity evaluation results of Benson et al., 1999 for a 4-week oral (rats, mice, hamsters) study on clinical chemistry/biochemical, and hepatic outcomes**

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

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| Study Citation: | Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | 4-week oral (rats, mice, hamsters) | | | | | |
| HERO ID: | 195107 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance. | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Low | × 2 | 6 | The species, strain, and sex of the animals were reported; however, source, health status, age, and starting body weight were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The number of animals per group (5 or 6 per time point of sacrifice) was less than typically used in studies of the same or similar type (i.e., subchronic-type studies). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was sensitive for the outcomes of interest (primarily hepatic and clinical chemistry outcomes, with mechanistic liver evaluation). | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | Details regarding the execution of the study protocol for outcome assessment, including time of assessments across study groups, were not fully reported. These reporting deficiencies may have a substantial impact on the results. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | Details regarding sampling of outcomes were not reported, which may have a substantial impact on the results. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | The negative control responses were reported and acceptable. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control responses were reported and acceptable. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and water intake were not reported and this may have a substantial impact on results because it was not reported if there were any palatability issues. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and health outcomes unrelated to exposure were not reported for each study group and this deficiency may have a substantial impact on results. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Low | × 1 | 3 | Statistical methods were not described clearly and this deficiency is likely to have a substantial impact on results. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Data for exposure-related findings were not clearly shown for each study group; however, results were briefly described in the text. This may have a substantial impact on the results. | |
| Overall Quality Determination [‡] | | Low | | 2.3 | | |
| Extracted | | Yes | | | | |
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Study Citation: Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report
 Data Type: 4-week oral (rats, mice, hamsters)
 HERO ID: 195107

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics <i>Metabolomics</i> , 10(6), 1293-1304 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | | | | | | |
| HERO ID: | 3487830 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | Commercial source was identified. | |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | Purity not reported. | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | Vehicle (corn oil) controls were used. | |
| | Metric 5: Positive Controls | Not Rated | NA | NA | Positive controls not used for liver toxicity. | |
| | Metric 6: Randomized Allocation | High | × 1 | 1 | Animals were randomly assigned to each dose group. | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation and storage were not described; however, omission of these details are unlikely to have a substantial impact on results (acute exposure). | |
| | Metric 8: Consistency of Exposure Administration | High | × 1 | 1 | Gavage volume was not excessive. | |
| | Metric 9: Reporting of Doses/Concentrations | High | × 2 | 2 | | |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | Acute studies are included; liver effects occur after acute exposure. | |
| | Metric 11: Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Two dose groups plus control. High dose chosen to induce mild to moderate adverse effects based on range-finding study. 30 animals received single dose and an additional 15 animals received a total of 3 once daily doses, which should be sufficient for the main purpose of this study (e.g., metabolomics). | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | High | × 2 | 2 | FDA colony; species, strain and starting age reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | | |
| | Metric 15: Number per Group | High | × 1 | 1 | 30 animals received single dose and an additional 15 animals received a total of 3 once daily doses | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Clinical chemistry and liver histopathology. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | 5/group used for clinical chemistry and histopathology. | |
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Study Citation: Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics *Metabolomics*, 10(6), 1293-1304

Data Type:
HERO ID: 3487830

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--|--|---------------------|------------------|-------|--|
| | Metric 19: Blinding of Assessors | Medium | × 1 | 2 | Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were well-described. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | |
| Overall Quality Determination [‡] | | High | | 1.2 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 11: Animal toxicity evaluation results of Civo et al., 1985 for a 4 week inhalation-liver toxicity (same as 4215910) study on hepatic outcomes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: Civo Institute Tno (1985). Fixed versus variable levels of exposure in inhalation toxicity testing with reference to the workplace studies with acetaldehyde and carbon tetrachloride | | | | | |
| Data Type: 4 week inhalation-liver toxicity (same as 4215910) | | | | | |
| HERO ID: 4215798 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by name. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Source was reported incompletely, but the omitted details are unlikely to have a substantial impact on results |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity such that effects likely due to test substance. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative controls were used. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not required. |
| Metric 6: | Randomized Allocation | High | × 1 | 1 | Computer randomization used for allocation. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Method and equipment of generation was reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations were reported. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Frequency and duration were reported. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | the number of groups and concentration spacing were reported and justified. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method were reported and appropriate. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The source, species, strain, sex, and initial body weight were reported. Health status and age were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Husbandry details were reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | The number of animals per group was appropriate. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was reported and appropriate. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently. |
| Metric 18: | Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for outcomes of interest. |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | Blinding not required. |
| Metric 20: | Negative Control Response | High | × 1 | 1 | Negative control responses were appropriate. |
| Domain 6: Confounding / Variable Control | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Respiratory rate was not reported but is not likely to have significant impact on results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | No health outcomes unrelated to exposure were reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were reported and appropriate. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | |
| Overall Quality Determination [‡] | | High | | 1.2 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

3 Other

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

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

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| Study Citation: | Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | inhalation and dw ADME studies (acute and subchronic) | | | | | |
| HERO ID: | 195107 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Studies of uptake, distribution and clearance in multiple species. CYP induction following in vivo exposure. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | | |
| | Metric 19: Blinding of Assessors | Medium | × 1 | 2 | Blinding was not reported, but outcomes were objective. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | respiratory rate was not reported and CCL4 is expected to be a respiratory irritant. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Not Rated | NA | NA | Not applicable to ADME data. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | ADME data were fully reported in data tables. | |
| Overall Quality Determination [‡] | | High | | 0.0 | | |
| Extracted | | No | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

4 Subchronic (30-90 days)

Table 13: Animal toxicity evaluation results of Adams et al., 1952 for a subchronic inhalation exposures (46 to 94 days) in rats study on renal, hepatic, nutrition and metabolic/adult exposure body weight, and cardiovascular outcomes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| Study Citation: Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
| Data Type: subchronic inhalation exposures (46 to 94 days) in rats | | | | | |
| HERO ID: 62373 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name and contaminants identified. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Both untreated and sham-treated control groups were used. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for this study type |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Study did not describe method of animal allocation |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal. |
| Metric 8: | Consistency of Exposure Administration | Low | × 1 | 3 | Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration. |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method. |
| Metric 10: | Exposure Frequency and Duration | Unacceptable | × 1 | 4 | Exposure frequencies ranged between 0.05 and 1 hour per day. This well below the daily duration typically used for subchronic toxicity evaluation. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 2 exposure concentrations were tested; the low concentration was tested at four different daily exposure durations (0.05 to 1 hr/day) |
| Continued on next page ... | | | | | |

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| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | subchronic inhalation exposures (46 to 94 days) in rats | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | Dynamic whole body chamber was used for vapor that may condense. | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, and source (in-house colony) were reported. Study reports choosing animals for the study based on health during pre-exposure observation period. Age and initial body weight were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions other than the exposure chamber were not reported. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Group sizes were 5 or 6/sex/group. This is consistent with recommendations for 28 day studies but less than recommended for subchronic studies. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was described, and outcomes included body weight and weights and histopathology of liver, kidney, lung, and heart. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | No inconsistencies in assessment of these endpoints were reported. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | All animals were evaluated for these endpoints. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | No subjective outcomes were evaluated. | |
| | Metric 20: Negative Control Response | Unacceptable | × 1 | 4 | Control responses were not reported. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food/water intake, and respiratory rate were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analysis was conducted and methodology described; the method did not account for multiple comparisons. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | All data were reported qualitatively without indication of which control group(s) was compared for statistical analysis. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.1 | | |
| Extracted | | No | | | | |

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 14: Animal toxicity evaluation results of Bruckner et al., 1986 for a study on renal and hepatic outcomes

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

Data Type:

HERO ID: 62379

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|------------------------------|--|---------------------|------|-------|--|
| | Metric 11: Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of dose groups and dose spacing were justified by the authors and considered adequate to address the purpose of the study (identifying NOAEL and LOAEL levels, with a focus on liver effects). |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance. |
| Domain 4: Test Organism | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and starting body weights (within a range) were reported.; the species and strain were appropriate. Animals were obtained from a commercial laboratory. Rats were described as adults (specific age not reported). Health status of the rats was not explicitly specified. These minor limitations are unlikely to substantially impact the study results. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Some husbandry conditions were specified (i.e. reverse light/dark conditions were reported) . The lack of information on other conditions (i.e. temperature, humidity) are considered minor uncertainties that are unlikely to have a substantial impact on the results (no indication that conditions were different among treated rats and controls). |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The number of animals per study group was reported.. However, only male rats were used; the number of animals used were 5 for acute and sub-acute studies, and 15-16 for the subchronic-duration study (compared to 10/sex/group used for standard 28-day and 90-day repeated-dose studies). |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment partially addressed the intended outcomes of interest. Liver and kidney effects were evaluated by examining limited clinical chemistry parameters, organ weights, and/or histopathological effects. Other common clinical chemistry parameters associated with liver and kidney function were not measured. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment protocols were described, and outcomes were assessed consistently across groups. Data for liver lesions were presented quantitatively as means (+/-SD) based on severity scores ranging from 0 to 8. |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Details regarding sampling for the outcomes of interest were reported, with minor limitations (e.g. number of histology slides evaluated not reported). Numbers of animals evaluated for specific endpoints were generally limited (5-9 males/group, even for the subchronic-duration study). |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | Histopathology slides were coded and examined in a single blind fashion. |

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| Study Citation: | Bruckner, JV; Mackenzie, WF; Muralidhara, S; Luthra, R; Kyle, GM; Acosta, D (1986). Oral toxicity of carbon tetrachloride: Acute, subacute, and subchronic studies in rats <i>Fundamental and Applied Toxicology</i> , 6(1), 16-34 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | | | | | | |
| HERO ID: | 62379 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | The biological responses of the control group were reported for most endpoints; however, liver histopathology data for control animals subjected to acute and sub-acute treatment were not shown (no effects were reported). Liver weight data were provided in the text for the control and high-dose groups only. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no reported differences in initial body weights among study groups. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | | |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Data were presented by exposure group for most endpoints. Liver histopathology data for the negative control group (acute and subacute studies) are not shown in the data tables. Liver weight data are provided for the control and high-dose groups only. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 15: Animal toxicity evaluation results of Hayes et al., 1986 for 90 day oral toxicity test in mice study on reproductive, hematological and immune, neurological, renal, hepatic, clinical chemistry/biochemical, mortality, nutrition and metabolic/adult exposure body weight, and respiratory outcomes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice <i>Fundamental and Applied Toxicology</i> , 7(3), 454-463 | | | | | |
| Data Type: 90 day oral toxicity test in mice | | | | | |
| HERO ID: 194400 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance source and lot number reported, but certification/analytical verification of identity was not. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance reported to be HPLC grade and >99% pure. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Both naive and sham-treated control groups were used. Sham-treated controls received vehicle. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for this study type. |
| Metric 6: | Randomized Allocation | Medium | × 1 | 2 | Study reports randomizing the mice but does not discuss the allocation to groups |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Study reports daily preparation of solution, but does not report storage. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Administration details are provided, including gavage volume and time of day of administration. No inconsistencies in exposures across groups were noted. |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | Dose reported in mg/kg bw; initial body weight not reported. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Animals gavaged daily for 90 days |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 4 nonzero doses ranging 100-fold were used. Effects were seen at all doses, so it is not clear that the lowest dose was low enough. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, lifestage, and source were reported and appropriate. Initial body weights were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were described and appropriate. |
| Metric 15: | Number per Group | High | × 1 | 1 | 20/sex/dose were tested |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Only liver and kidney were examined microscopically |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | No inconsistencies in outcome assessment were noted by the authors. |

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| Study Citation: | Hayes, JR; Condie, LW; Borzelleca, JF (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice <i>Fundamental and Applied Toxicology</i> , 7(3), 454-463 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | 90 day oral toxicity test in mice | | | | | |
| HERO ID: | 194400 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Mortality, body weight, histopathology, and organ weights were reportedly evaluated in all animals; hematology and serum chemistry evaluated in subgroups of 8-10/sex/dose due to low blood volume of mice. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Although study reports observing animals for signs of intoxication, no results were reported, so lack of blinding would not be of concern. No other subjective outcomes were evaluated | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Responses of both naive and vehicle controls were reported for all endpoints other than hematology. Responses appeared to be as expected and without excessive variability. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food and water intake were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Study reports that there were no compound-related deaths, but there were several mortalities in the vehicle control and high dose groups (in males) and in vehicle control and all exposure groups (in females); these were presumably gavage errors. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were reported and appropriate to the data. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | There are unexplained inconsistencies in the numbers of animals exposed and evaluated for histopathology vs the numbers of animals for which histopathology results are reported. Although the authors report that histopathology was evaluated in all control and exposed mice, results are reported for only 10/sex/dose (vs 20/sex/dose exposed and evaluated for organ weights). The authors do not explain this apparent discrepancy. | |
| Overall Quality Determination [‡] | | High | | 1.7 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 16: Animal toxicity evaluation results of Allis et al., 1990 for a 12-week oral study on hepatic, nutrition and metabolic/adult exposure body weight, and clinical chemistry/biochemical outcomes

| Study Citation: | Allis, JW; Ward, TR; Seely, JC; Simmons, JE (1990). Assessment of hepatic indicators of subchronic carbon tetrachloride injury and recovery in rats Fundamental and Applied Toxicology, 15(3), 558-570 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | 12-week oral | | | | | |
| HERO ID: | 194565 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified definitively. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity and grade were not reported and there was no analysis conducted for measurement of impurities, if present. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | A concurrent negative control group was used and was appropriate. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive control is not indicated by the study type. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study authors did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | The study authors stated that new gavage solutions were used daily and were prepared weekly; however, the procedures for preparing the solutions in vehicle (corn oil) were incompletely reported and storage conditions were not reported. Deficiencies in reporting may have a substantial impact on results. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details on exposure administration were reported, including consistent dosing volumes, and exposures were administered consistently across study groups in a scientifically sound manner (dose volume of 2 mL/kg was acceptable). | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | The administered doses were reported without ambiguity. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency and duration were reported and were appropriate for the study type and outcomes of interest. In this subchronic study, animals were gavaged 5 days per week for 12 weeks. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Although two quantitative dose groups (20 and 40 mg/kg/day) were used, there were deficiencies in the dose spacing. Adverse effects, including liver histopathology, clinical chemistry, and reduced body weight gain, were observed at both doses and, in some cases, there were few differences between the two dose groups (e.g., histopathology incidence). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported (gavage) and suited to the test substance. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|---|
| Domain 4: Test Organism | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal characteristics were reported (source, species, strain, sex, age, starting body weight); however, health status at the start of the study was not reported. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Most husbandry conditions were reported and were adequate and similar for all groups. |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Groups were subdivided for some evaluations, resulting in a lower number of animals per group than is typical. For example, from the 24 animals/dose level, 6 animals each were terminated on respective days 1, 8, 15, and 22 post-exposure for evaluation of hepatic cytochrome P450, serum chemistry, and light microscope histopathology, resulting in only 6 animals/dose group evaluated for these endpoints. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest, which were primarily effects on the liver. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups. |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Details regarding sampling for the outcomes of interest were reported and were adequate. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | No subjective outcomes were reported. Blood samples were assayed commercially and histopathology was not described as a re-evaluation so I scored this metric as not applicable. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control response was adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no reported differences among the study groups in initial body weight or food or water intake that could influence the outcome assessment. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted. |
| Domain 7: Data Presentation and Analysis | | | | | |

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

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

| Study Citation: | Condie, LW; Laurie, RD; Mills, T; Robinson, M; Bercz, JP (1986). Effect of gavage vehicle on hepatotoxicity of carbon tetrachloride in CD-1 mice: corn oil versus Tween-60 aqueous emulsion Toxicological Sciences, 7(2), 199-206 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | 90-day oral | | | | | |
| HERO ID: | 60712 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified definitively. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance, including manufacturer and lot number, was reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity was reported (98.2%) and impurities (chloroform, 1.8%) were identified. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using appropriate concurrent negative control groups (corn oil and Tween-60). | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive control is not indicated for the study type. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study authors did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | The study authors did not report preparation and storage conditions, including how often test substance was prepared and under what conditions the test substance was stored. Deficiencies in reporting may have a substantial impact on results. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration were reported and exposures were administered consistently across study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Administered doses were reported without ambiguity. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency and duration of exposure were reported and appropriate for the study type and outcomes of interest. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups and spacing were reported and considered adequate for the purpose of the study. Selected concentrations were not justified by the study authors but the selected doses appear acceptable. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and these were suited to the test substance. | |
| Domain 4: Test Organism | | | | | | |
| Continued on next page ... | | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The animal species, strain, and sex were reported; however, age, starting body weight, and health status were not reported. The test species was obtained from a commercial source and was an appropriate model for evaluation of the outcomes of interest. The reporting deficiencies are unlikely to have a substantial impact on results. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were reported (e.g., temperature, humidity, light-dark cycle) and were adequate and the same for control and exposed populations. |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per study group was reported and appropriate for the study type. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | The outcome assessment protocol was reported; however, the descriptions of sampling of blood for serum enzymes do not clearly indicate when blood was collected from the animals. |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Details regarding sampling for the outcomes of interest were reported by the study authors and the study used adequate sampling for the outcomes of interest (e.g., adequate number of animals from each group). |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | The study did not report evaluation of subjective outcomes except histopathology. According to the criteria, however, this metric is not rated/applicable for initial histopathology review. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control response was adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no confounding variables among the study groups that could influence the outcome assessment. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on attrition or health outcomes unrelated to exposure were not reported because only substantial differences among groups were noted. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The statistical methods were clearly described and appropriate for the data set. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for exposure-related findings were presented for all outcomes by exposure group and sex with quantal and/or continuous presentation and description of severity scores. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------|---------------------|------|-------|------------------------|
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 18: **Animal toxicity evaluation results of Benson et al., 1999 for a 12-week inhalation (rats, mice, hamsters) study on clinical chemistry/ biochemical, and hepatic outcomes**

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|---|
| Study Citation: Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
| Data Type: 12-week inhalation (rats, mice, hamsters) | | | | | |
| HERO ID: 195107 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified definitively. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source and batch/lot number of the test substance was not reported. The omitted details are likely to have a substantial impact on the results. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and grade of the test substance were not reported and this may have a substantial impact on the results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | The study authors reported using a concurrent negative control group but details regarding the negative control group were not reported and the lack of details may have a substantial impact on the results. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control is not indicated for the study type. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study authors did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | The study authors did not describe the test substance preparation and storage conditions. The reporting deficiencies are likely to have a substantial impact on results. |
| Metric 8: | Consistency of Exposure Administration | Unacceptable | × 1 | 4 | Critical exposure details, including the methods for generating atmosphere in inhalation chambers, were not reported. |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Target concentrations were reported; however, actual concentrations were not reported and there was no indicated that test concentrations were monitored/measured during the inhalation exposures. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure duration and frequency (6 hours/day, 5 days/week, for 1, 4, or 12 weeks) were reported and were suited to the study type and outcomes of interest (clinical chemistry, liver histopathology, and liver proliferation/mechanistic [via BrdU labeling]). |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups and dose spacing (0, 5, 20, 100 ppm) were reported and were relevant for the assessment. Although not justified, it appears that concentrations were based on results reported in previous studies. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance. |

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| Study Citation: | Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
|--|---|--|------|-------|--|--|
| Data Type: | 12-week inhalation (rats, mice, hamsters) | | | | | |
| HERO ID: | 195107 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The species, strain, and sex of the animals were reported; however, source, health status, age, and starting body weight were not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations. | |
| Metric 15: | Number per Group | Medium | × 1 | 2 | The number of animals per group (5 or 6 per time point of sacrifice) was less than typically used in studies of the same or similar type (i.e., subchronic-type studies). | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was sensitive for the outcomes of interest (primarily hepatic and clinical chemistry outcomes, with mechanistic liver evaluation). | |
| Metric 17: | Consistency of Outcome Assessment | Low | × 1 | 3 | Details regarding the execution of the study protocol for outcome assessment, including time of assessments across study groups, were not fully reported. These reporting deficiencies may have a substantial impact on the results. | |
| Metric 18: | Sampling Adequacy | Low | × 1 | 3 | Details regarding sampling of outcomes were not reported, which may have a substantial impact on the results. | |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | | |
| Metric 20: | Negative Control Response | High | × 1 | 1 | The negative control responses were reported and acceptable. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| Metric 21: | Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Respiratory rate measurement was not reported. This is considered to have a substantial impact on results since CCl4 is a potential respiratory irritant. | |
| Metric 22: | Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and health outcomes unrelated to exposure were not reported for each study group and this deficiency may have a substantial impact on results. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| Metric 23: | Statistical Methods | Low | × 1 | 3 | Statistical methods were not described clearly and this deficiency is likely to have a substantial impact on results. | |
| Metric 24: | Reporting of Data | Low | × 2 | 6 | Data for exposure-related findings were not clearly shown for each study group; however, results were briefly described in the text. This may have a substantial impact on the results. | |
| Overall Quality Determination[‡] | | Unacceptable** → Low[§] | | | 2.4 | |
| Extracted | | Yes | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
| Data Type: Inhalation and dw ingestion studies (1, 4 and 12 weeks) | | | | | |
| HERO ID: 195107 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified by chemical name. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | No details were provided on them source of the test substance. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative air and dw controls. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls are not generally included in studies of liver toxicity. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Inhalation exposure details were provided in the ADME study description (method of vapor generation was described and appropriate). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Actual concentrations were not reported. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Continuous exposure for dw; 6hour/day 5 days/wk, for 1, 4 or 12 weeks. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | 2-3 groups plus control; doses were not justified , but dose response relationships were apparent. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Dynamic whole-body chambers. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Rat, mouse and hamster species, strain and age were reported in the ADME study, Obtained from commercial source. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Adequate husbandry conditions as described in ADME studies. |
| Metric 15: | Number per Group | Medium | × 1 | 2 | 5-6/group for most endpoints (10/group for serum chemistry at 12 weeks); adequate for statistics. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome methods were sensitive for hepatotoxicity (serum chemistry, histopath. and hepatocellular replication). |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | |
| Metric 18: | Sampling Adequacy | High | × 1 | 1 | |

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| Study Citation: | Benson, JM; Springer, DL (1999). Improved risk estimates for carbon tetrachloride. Final report | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Inhalation and dw ingestion studies (1, 4 and 12 weeks) | | | | | |
| HERO ID: | 195107 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 19: Blinding of Assessors | Medium | × 1 | 2 | Blinding was not reported ; however, outcomes were objective. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | No incidence of hepatocellular necrosis in controls. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Respiratory rate was not measured; CCl4 is anticipated to be a respiratory irritant. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all time points and exposure groups. | |
| Overall Quality Determination [‡] | | High | | 1.6 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

5 Chronic (>90 days)

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--|---|---------------------|------------------|-------|---|
| Study Citation: Nagano, K; Sasaki, T; Umeda, Y; Nishizawa, T; Ikawa, N; Ohbayashi, H; Arito, H; Yamamoto, S; Fukushima S (2007). Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice Inhalation Toxicology, 19(13), 1089-1103 | | | | | |
| Data Type: 2-year bioassay | | | | | |
| HERO ID: 194127 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified definitively. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported, including manufacturer; however, the batch/lot number was not reported, although identity was verified by analytical means (gas chromatography) by the study laboratory. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The test substance purity and composition were such that any observed effects were highly likely to be due to the test substance itself. The purity was reported as 99.8% and other components were identified with purities provided. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using an appropriate concurrent negative control group. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive control group is not indicated by study type. |
| Metric 6: | Randomized Allocation | Medium | × 1 | 2 | The study reported that the animals were divided by stratified randomization; however, there were minor limitations in the allocation method due to use of a non-random component (body weight-matched grouping). |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation methods were reported and appropriate for the test substance. The method and equipment for generating the test substance as a vapor were reported and appropriate. Although not reported, test substance storage conditions were considered appropriate based on observation of stability of the test substance before and after use by gas chromatography and infrared spectrometry analysis. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure methods were reported and exposures were administered consistently for the study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations were reported without ambiguity. Mean measured concentrations were reported based on chamber concentrations monitored every 15 minutes during the exposures by gas chromatography. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency and duration of exposure were reported and were appropriate for the study type and outcomes of interest. |

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| Study Citation: | Nagano, K; Sasaki, T; Umeda, Y; Nishizawa, T; Ikawa, N; Ohbayashi, H; Arito, H; Yamamoto, S; Fukushima S (2007). Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice Inhalation Toxicology, 19(13), 1089-1103 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | 2-year bioassay | | | | | |
| HERO ID: | 194127 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 11: Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | The number of exposure groups and concentration spacing were justified; however, the highest concentration (125 ppm) resulted in early mortality of most animals. Therefore, there were an insufficient number of animals in this group for statistical analysis of some endpoints, including terminal body weights, organ weights, clinical chemistry, and urinalysis. Two lower concentrations, 5 and 25 ppm, were also included in the study and a sufficient number of animals survived the duration of exposure for statistical analysis on the same endpoints. | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and suited to the test substance. | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The test animal source, species, strain, sex, age, and starting body weight were reported; however, health status was not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were reported, including temperature, humidity, and light-dark cycle, and were adequate and no differences were reported for the test substance-exposed and control groups. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per study group (50/sex/group) was reported and appropriate for the study type. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups using the same protocol. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | No subjective outcomes were reported so I considered this metric not applicable. Blood samples were analyzed automatically and histopathology was not described as a re-evaluation. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control were adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | There were no confounding differences among the study groups in initial body weight. Respiratory rate, however, was not reported. | |
| Continued on next page ... | | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among study groups were noted. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The statistical methods were clearly described and appropriate for the data. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | |
| Overall Quality Determination [‡] | | High | | 1.2 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 21: Animal toxicity evaluation results of Nagano et al., 2007 for a 13-week inhalation study in rats and mice study on renal, hepatic, hematological and immune, clinical chemistry/biochemical, and body weight outcomes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: Nagano, K; Umeda, Y; Saito, M; Nishizawa, T; Ikawa, N; Arito, H; Yamamoto, S; Fukushima, S (2007). Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice Journal of Occupational Health, 49(4), 249-259 | | | | | |
| Data Type: 13-week inhalation study in rats and mice | | | | | |
| HERO ID: 194237 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Analytical-grade CCl4 |
| Metric 2: | Test Substance Source | High | × 1 | 1 | source clearly identified.. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | purity specified (98%); each lot analyzed for stability and purity. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | used appropriate concurrent negative control group (clean air) under the same conditions as treated groups. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | this metric is not rated/ applicable because a positive control is not indicated by this study type. |
| Metric 6: | Randomized Allocation | Medium | × 1 | 2 | Animals allocated using stratified randomization into weight-matched groups |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | The method and equipment used to generate the test substance as a vapor were reported and appropriate. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration were clearly reported and were consistent across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Target and analytical concentrations were reported and appropriate. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency and duration of exposure were reported and appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups and dose/concentration spacing were justified by study authors |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The test animal was obtained through the reported commercial source. The test animal species, strain, sex, and age were specified. Starting body weight was not reported, but the authors note that animals were randomized into weight-matched groups. The authors also don't explicitly mention health status of the animals. These omissions are unlikely to have a substantial impact on results. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | All husbandry conditions were reported and adequate. Conditions were the same for control and treated groups. |

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| Study Citation: | Nagano, K; Umeda, Y; Saito, M; Nishizawa, T; Ikawa, N; Arito, H; Yamamoto, S; Fukushima, S (2007). Thirteen-week inhalation toxicity of carbon tetrachloride in rats and mice Journal of Occupational Health, 49(4), 249-259 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | 13-week inhalation study in rats and mice | | | | | |
| HERO ID: | 194237 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcomes of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Details regarding sampling for the outcomes of interest were reported and adequate. Endpoints were evaluated in an adequate number of animals in each group. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Most outcomes were not subjective; this metric is not rated/applicable for initial histopathology review. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control groups were adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | There was a lack of reporting of respiratory rates; but this lack of reporting is not likely to have a significant impact on results. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on attrition and health outcome unrelated to exposure for each study group were not reported; the lack of reporting is unlikely to have a substantial impact on results. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were clearly described and appropriate for datasets | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for exposure-related findings were presented for all outcomes by exposure group and sex | |
| Overall Quality Determination [‡] | | High | | 1.2 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 22: Animal toxicity evaluation results of Adams et al., 1952 for a 6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys study on renal, hepatic, respiratory, cardiovascular, hematological and immune, nutrition and nutrition and metabolic/adult exposure body weight outcomes

| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | 6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by unambiguous name and contaminants identified. | |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test substance was reportedly a commercial product but specific source was not reported. Infrared absorption spectroscopy used to verify identity and identify contaminants. | |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Test substance purity not reported, but paper reports purification of commercial product by redistillation and confirmation of identity by infrared absorption spectroscopy. Minor contaminants were identified at low (= 0.05%) concentrations. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Both untreated and sham-treated control groups were used. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls not typical for this study type | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Study did not describe method of animal allocation | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Method of vapor generation was incompletely reported (equipment not specified; temperature used to achieve vaporization was not reported) but there is no reason to believe there would be an impact on animal exposure, as vapor concentrations were reportedly analyzed regularly and within 10% of nominal. | |
| Metric 8: | Consistency of Exposure Administration | Low | × 1 | 3 | Exposures at different concentrations were administered for different durations, making it difficult to discern effects of changing duration from effects of changing concentration. | |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Air concentrations were reported, but it is not clear whether these were nominal or actual concentrations. Analysis of chamber concentrations was by combustion analysis, which is likely an insensitive method. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposures were 7 hr/day, 5 d/wk for durations ranging up to about 6 months. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | 7 exposure concentrations were tested in rats and guinea pigs, with an overall range of 80-fold. 5 concentrations with a range of 20 fold were tested in rabbits and monkeys. Concentrations were sufficient to identify effect levels. | |
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| Study Citation: | Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66 | | | | | |
|--|--|---------------------|------|------------------|---|--|
| Data Type: | 6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys | | | | | |
| HERO ID: | 62373 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | Dynamic whole body chamber was used for vapor that may condense. | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, and source (in-house colony) were reported. Study reports choosing animals for the study based on health during pre-exposure observation period. Age and initial body weight were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions other than the exposure chamber were not reported. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Group sizes were 15/sex for rats, 8/sex for guinea pigs, 2/sex for rabbits, and 2 monkeys. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was described, and outcomes included body weight and weights and histopathology of liver, kidney, lung, heart, and spleen. Authors note that limited blood chemistry and hematology endpoints were assessed "in many cases". | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | Authors note that limited blood chemistry, liver lipids, and hematology endpoints were assessed "in many cases" but do not specify which groups were evaluated. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | Details regarding outcome sampling were not reported. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Endpoints were not subjective. | |
| | Metric 20: Negative Control Response | Low | × 1 | 3 | Control responses were reported only for body weights and organ weights. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food/water intake, and respiratory rate were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analysis was conducted and methodology described; the method did not account for multiple comparisons. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | All data were reported qualitatively without indication of which control group(s) was compared for statistical analysis. | |
| Overall Quality Determination [‡] | | Medium | → | Low [§] | 2.1 | |
| Extracted | | Yes | | | | |
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Study Citation: Adams, EM; Spencer, HC; Rowe, VK; Mccollister, DD; Irish, DD (1952). Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals Archives of Environmental and Occupational Health, 6(1), 50-66
 Data Type: 6 month inhalation exposures in rats, guinea pigs, rabbits, and monkeys
 HERO ID: 62373

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "Limited (predominantly qualitative) reporting of results, varying exposure durations."

6 Genetic toxicity studies

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

| Study Citation: | R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compunds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357 | | | | |
|-------------------------------------|---|---------------------|------------------|-------|--|
| Data Type: | DNA adducts (32P-postlabeling assay) | | | | |
| HERO ID: | 6146 | | | | |
| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified by chemical name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Manufacturer was reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of test substance were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent vehicle controls were used (same injection volume). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation in corn oil was described. Storage was not reported; however, only a single injection was used. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was administered consistently. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Information was provided to allow calculation of dose (% v/v, ml/kg bw). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single dose was adequate for the outcome of interest. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single dose group; level was not justified. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Route and method were suited to the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and age were reported. The test animal was obtained from a commercial source. Body weight and health status were not reported. Mice were described as retired breeders (10-12 months old). |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | 3-4/group was adequate for the outcome of interest. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment method was reported and sensitive for the outcome of interest. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome was assessed consistently across groups. |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |

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| Study Citation: | R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compounds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | DNA adducts (32P-postlabeling assay) | | | | | |
| HERO ID: | 6146 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response appears adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | The lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were described and appropriate. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were fully reported across timepoints. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 24: **In vitro** evaluation results of Tafazoli et al., 1988 for DNA damage (Comet assay)

| Study Citation: | M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential <i>Mutagenesis</i> , 13(2,2), 115-126 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | DNA damage (Comet assay) | | | | | |
| HERO ID: | 194476 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified as Carbon tetrachloride and CASRN was provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source was reported | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity 99% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent media-alone controls were used (no solvents were used during test preparation) | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Appropriate positive controls for conditions with and without metabolic activation were used. | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were clearly described and appropriate for the outcome of interest. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Information on test substance preparation was adequately described. Methods were employed (use of sealed bottles) to prevent evaporation during the process. The duration of the test substance preparation however was lengthy (48hours, shaking at 37 degrees), and the rationale for this and the potential impact on stability was not discussed. There is further uncertainty about the stability of the test substance due to lack of DMSO as a solvent. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Consistent application methods are inferred from the text. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Initial Test substance concentrations (3) were reported without ambiguity. Analytical concentrations measured after the media preparation procedure were not reported. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was appropriate for the outcome of interest | |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | The concentrations chosen (three) were based on previous experiments evaluating micronuclei. The highest concentration was not cytotoxic in previous experiments and no positive response was observed. Evaluating the highest concentration resulting in some cytotoxicity would be warranted. | |
| Metric 13: | Metabolic Activation | Low | × 1 | 3 | The study included conditions of metabolic activation (s9), however, the source and method of preparation of the S9 mix were not provided. | |

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| Study Citation: | M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential <i>Mutagenesis</i> , 13(2,2), 115-126 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA damage (Comet assay) | | | | | |
| HERO ID: | 194476 | | | | | |
| Domain | Metric | Rating [‡] | MWF* | Score | Comments ^{††} | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (primary human lymphocytes) was appropriate. Descriptive information on the source and method of isolation was provided. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Two replicates ("parallel cultures" from each donor) were utilized. This is considered to be somewhat lacking. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the outcome of interest | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across exposure groups | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | Sampling was generally adequate for the outcome of interest (100 cells total from two cultures). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No confounding variables in the test design or procedures were identified. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Confounding variables on outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical methods were clearly stated and appropriate for the outcome of interest. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Circumstances yielding a positive result were described. Positive results were based on reaching statistical significance. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Concurrent cytotoxicity evaluations were not done with this experiment, however cytotoxicity was assessed both in a preliminary range-finding study, and was also evaluated in micronuclei tests that were performed in cells from the same donors using the same concentrations tested for this outcome. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data was adequately presented across all groups | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |
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Study Citation: M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential *Mutagenesis*, 13(2,2), 115-126

Data Type: DNA damage (Comet assay)

HERO ID: 194476

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Outcomes were assessed using GLC/MS. The study indicated that this was the only technique that could be used due to the low product yield from the reaction mixture, which prevented use of other methods like NMR. |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | It is inferred from the text that the same outcome assessment was applied to test and control groups |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for the study design |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study design |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Not Rated | NA | NA | Not applicable for the study design |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Not Rated | NA | NA | Not applicable for the study design |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Not applicable for the study design |
| | Metric 23: Data Interpretation | Not Rated | NA | NA | Not applicable for the study design - Study design did not involve positive or negative findings, only presence of reaction products. |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | Not applicable for the study design |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | GLC/MS profiles were included in the report, but profiles from control samples were not provided. |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 26: **In vitro** evaluation results of Amacher et al., 1983 study on mammalian cell transformation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|--|
| Study Citation: D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296 | | | | | |
| Data Type: Mammalian cell transformation for CCl4 | | | | | |
| HERO ID: 194590 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Appropriate concurrent negative control groups were included (DMSO). |
| Metric 5: | Positive Controls | High | × 2 | 2 | Positive controls were tested concurrently with each test substance. The identity of each positive control was reported (ethyl methanesulfonate and benzo[a]pyrene) and appropriate. Positive controls yielded positive results. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay methods and procedures were adequately described. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Test substance preparation was reported. Test substance storage was not reported; however, solutions were prepared immediately before administration (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The doses are reported without ambiguity. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number of exposure groups and dose spacing was reported and appropriate for this assay. |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to this study design. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The identity and method of isolation of the primary Syrian golden hamster embryo cells used here were reported and appropriate for the outcome of interest. |
| Metric 15: | Number per Group | High | × 1 | 1 | The experiment was conducted with 30 wells per dose level per test substance. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology is appropriate for the outcome of interest. |

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| Study Citation: | D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Mammalian cell transformation for CCl4 | | | | | |
| HERO ID: | 194590 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this endpoint. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to this study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each treatment group. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | This metric is not applicable to the study design. Statistical analysis was not conducted on these data; any transformed colonies > 0 was considered a positive result. The raw data do not allow for an independent analysis because the data yielded from multiple doses per test substance were apparently pooled. | |
| | Metric 23: Data Interpretation | Not Rated | NA | NA | Scoring and evaluation criteria for assessing transformed colonies were cited to other publications. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | A preliminary toxicity assay was conducted to assess cytotoxicity levels. The doses for the mutagenicity assay were selected so that 50-90% survival was permitted. It is unclear what the methodology for assessing cytotoxicity was, and it is unclear whether cytotoxicity was assessed concurrently with the transformation assay. | |
| | Metric 25: Reporting of Data | | × 2 | NA | Raw data yielded from multiple dose levels per test substance were apparently pooled. Therefore, the data reporting is inadequate. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.5 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures Journal of Applied Toxicology, 8(4,4), 243-248 | | | | | |
| Data Type: Ames assay _CCL4 and chloroform | | | | | |
| HERO ID: 194606 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified by established nomenclature; no CASRNs were provided |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The test substance source was not identified |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not reported |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | The study reports using negative controls, but it is unclear if they were untreated or solvent controls. It was reported however, that separate experiments with the solvent (methanol) were performed and that it was both nontoxic and nonmutagenic. |
| Metric 5: | Positive Controls | Low | × 2 | 6 | Appropriate positive controls were included for studies with (2-anthramine) and without (sodium azide, 2-nitrofluorene, and 9-aminoacridine) activation. However, the positive control responses were not reported. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | The assay procedure was cited to another study with some details provided (plate incorporation assay); assay is standard for evaluating the outcome of interest; |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Preparation of test substances was adequately described, however details of mixing, stability and considerations of volatility were not discussed. Plate incorporation method uses; this method does not mitigate volatilization; however, positive results were seen so levels in medium were high enough to induce effect. Test substance storage was not reported |
| Metric 9: | Consistency of Exposure Administration | Not Rated | NA | NA | Exposure administration was not described in detail, studies were performed according to another publication. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations can be estimated from the figures provided. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration (2 days) was reported and appropriate for the study design |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | Six (CCL4) or five (chloroform) concentration groups were tested. No justification for the concentrations tested were provided. It is unknown whether concentrations were adequate or tested up to the level of cytotoxicity. |
| Continued on next page ... | | | | | |

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| Study Citation: | M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures Journal of Applied Toxicology, 8(4,4), 243-248 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Ames assay _CCL4 and chloroform | | | | | |
| HERO ID: | 194606 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | Aroclor-1254 induced rat livers were commercially obtained. 0.5 mL S9 was used for metabolic activation. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (strains of <i>S. typhimurium</i>) are routinely used for the outcome of interest. The source (B.Ames) was reported. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Tests without activation were performed on 4 strains of <i>S. typhimurium</i> . With activation, only two strains were used for CCL4. Generally, these numbers are less than typically recommended number (5) for a mutagenicity study; <i>E. coli</i> was also not included. Each concentration was tested in duplicate (two plates) and two or three independent experiments performed at different times/days. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment methodology was not reported, but is assumed to have been done according to the publication(s) cited which describe the assay procedure. | |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment methodology was not reported, but is assumed to have been done according to the publication(s) cited which describe the assay procedure. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | NA for this study type | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable to this study type | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial batch/lot number of organisms used per group was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | The study indicates statistical analysis was performed using Student's t-test and results that were significant were reported in the text. SD or SE were not provided, preventing independent statistical analysis. | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Scoring/evaluation criteria were not reported, but it appears that statistical analysis was the basis for positive/negative conclusions | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Cytotoxicity was not included in the study design or discussed in the text and it could not be determined that cytotoxicity was accounted for in the interpretation of study results. | |
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Study Citation: M. M. Varma, F. R. Ampy, K. Verma, W. W. Talbot (1988). In vitro mutagenicity of water contaminants in complex mixtures *Journal of Applied Toxicology*, 8(4,4), 243-248
 Data Type: Ames assay _CCL4 and chloroform
 HERO ID: 194606

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 28: **In vitro** evaluation results of Wangenheim et al., 1988 for a study on mouse lymphoma mutagenicity assay

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds <i>Mutagenesis</i> , 3(3,3), 193-205 | | | | | |
| Data Type: Mouse Lymphoma Mutagenicity Assay - CCl4 | | | | | |
| HERO ID: 194626 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Supplied by E. Merck (FRG). |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Study notes that chemicals were of the highest purity available; however, it does not report the actual purity of CCl4. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Six replicate solvent control agar plates were used for each chemical tested. |
| Metric 5: | Positive Controls | High | × 2 | 2 | 4-Nitroquinoline-N-oxide was tested without metabolic activation and benzo[a]pyrene was tested with metabolic activation. Both gave expected results. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay performed according to Clive et al., 1979 with some modifications that were briefly described. Some details were lacking (pre- and post-incubation temperatures, humidity, etc.) |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Not Rated | NA | NA | Methods were cited to another publication (Clive et al., 1979), and no additional details were provided regarding test substance preparation or storage. Lack of storage information is not likely to impact results as the test was performed for a short duration (4-hour exposure). |
| Metric 9: | Consistency of Exposure Administration | Not Rated | NA | NA | Exposure methods were cited to another publication (Clive et al., 1979), and no additional details were provided regarding the application of CCl4. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported clearly as 0, 1.030E-3, 3.100E-3, and 4.130E-3 mol/L. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Treatment with the test substance was 4 hours and appropriate to the outcome of interest |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Justification for using 3 test concentrations and the concentrations chosen was briefly reported. 'Compounds were generally tested up to a concentration that reduced total growth to 10-20% of the solvent control except in those cases when low solubility precluded the achievement of toxic concentrations'. Four test concentrations are recommended for this assay type. |

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| Study Citation: | J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds <i>Mutagenesis</i> , 3(3,3), 193-205 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Mouse Lymphoma Mutagenicity Assay - CCl4 | | | | | |
| HERO ID: | 194626 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | CCl4 was only tested in the presence of metabolic activation. Aroclor 1254 pretreated male Sprague-Dawley rat liver homogenate (S9) was prepared in the lab or purchased from Litton Bionetics Inc., Kesington, MD, USA and was prepared according to Garner et al., 1972. Cofactors (not further specified) from Sigma Chemical Co. were added as described in Clive et al., 1979. Concentration or volume of S9 mix used was not specified. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | Mouse lymphoma heterozygous L5178Y TK +/- 3.7.2.C cells were used and appropriate. Cells were obtained from Dr. Donald Clive of Burroughs Wellcome Co., Research Triangle Park, NC, USA. | |
| | Metric 15: Number per Group | High | × 1 | 1 | 3 replicates per treated culture were used and appropriate for a mammalian cell mutation assay. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology (manual and automatic colony counting) was reported and appropriate. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Referred to Clive et al., 1979. Assays where an irregular or absence of dose response occurred were retested. Approximately 20 chemicals were retested, but these were not specified. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design, as no subjective outcomes were assessed. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each replicate or group. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each replicate or group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The results were subject to a statistical analysis. Tests for normal distribution of colony number (according to Shapiro and Wilk, 1965) were performed; ANOVA, and then pairwise two-tailed Student's t-test was performed to compare treated replicates versus the solvent control replicates. Table of results also provided for each concentration tested. | |

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| Study Citation: | J. Wangenheim, G. Bolcsfoldi (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds <i>Mutagenesis</i> , 3(3,3), 193-205 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Mouse Lymphoma Mutagenicity Assay - CCl4 | | | | | |
| HERO ID: | 194626 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Evaluation criteria were briefly described and appropriate. Results were deemed positive if mutagenic activity was dose-related and statistically significant (1% or greater) when total growths were 10% or higher. Alternative interpretation using a 2-fold or greater increase in mutation frequencies with a 10% or higher growth was also considered. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Total growth (suspension growth x cloning efficacy) was evaluated based on methods described in Clive et al., 1979. Total growth was 20% at highest concentration tested., consistent with target survival for highest concentration (10-20%) reported by study authors | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Table of results reported the total growth, mutation frequency, and mutation index with results of statistical analysis for all concentrations of CCl4 tested. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 29: **In vitro** evaluation results of Watanabe et al., 1998 for a study on bacterial reverse mutation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: K. Watanabe, K. Satamoto, T. Sasaki (1998). Comparisons on chemically-induced mutation among four bacterial strains, <i>Salmonella typhimurium</i> TA102 and TA2638, and <i>Escherichia coli</i> WP2/pKM101 and WP2 <i>uvrA</i> /pKM101: Collaborative study II Mutation Research, 412(1,1), 17-31 Data Type: Bacterial reverse mutation for Perc HERO ID: 194631 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name. A CASRN was also provided. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was reported. Although a batch/lot number was not provided, it was indicated that the same lot of each chemical was used for all experiments. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The study did not indicate the purity of the test substance; however, it was indicated that chemicals used in the study were of the 'highest purity.' It is expected that observed effects are due to the test substance itself; the omission of the specific purity of the test substance is not likely to impact the study results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | The study used negative controls; all conditions except exposure appeared to be equal. It was not explicitly specified (but it was inferred from the study) that the negative control was a solvent-only (DMSO-only) control. |
| Metric 5: | Positive Controls | Medium | × 2 | 4 | A concurrent positive control was reportedly used (2-aminoanthracene in the presence of activation). Although the study noted that increased numbers of revertant colonies were observed in all strains with the positive controls in all experiments, positive control data were not shown. This omission is unlikely to have a substantial impact on results. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were briefly described, and partially cited to another publication (Watanabe et al., 1996). |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was inferred from the test (i.e., dissolved in DMSO), but storage was not reported (unlikely to affect results owing to the short duration of the study). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity (Appendix A). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The duration of the study was reported and consistent with other studies of this type. |
| Continued on next page ... | | | | | |

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| Study Citation: | K. Watanabe, K. Satamoto, T. Sasaki (1998). Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: Collaborative study II Mutation Research, 412(1,1), 17-31 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Bacterial reverse mutation for Perc | | | | | |
| HERO ID: | 194631 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | | × 1 | NA | The study used 6 doses plus controls (5 analyzable doses in most strains owing to toxicity). The doses selected appeared appropriate to evaluate dose-response and the test was conducted up to a dose that caused cytotoxicity. | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | The study authors reported that exposures were conducted in the presence of metabolic activation; the source and concentration in final culture were described. The type (rat, mouse, hamster) of S9 was not reported, but this is unlikely to impact the study results. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Not Rated | NA | NA | The study indicated that details associated with the bacterial strains were described in another publication (Watanabe et al., 1996). The characteristic properties of bacterial strains used were reported in the introduction of the study. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The study indicated that there were three plates per dose. In addition, it was noted that the test chemical was subjected to at least two independent experiments in two laboratories. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology (counting of revertant colonies after 48 hours incubation) addressed or reported the intended outcome of interest (mutagenicity). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | | × 1 | NA | This metric is not applicable to the study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | The study explicitly specified that precautions were taken to ensure that there were no differences among the initial study parameters (the bacterial strains used from a central source, the same lot of test substance used in all experiments). | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported (not likely to substantially impact the study results). | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The study indicates that data were analyzed using a linear regression test (based on a recommendation for this type of analysis from a cited publication) and using a significance level of 1%. Data provided in the study were not amenable to independent analysis (mean with no measure of variance provided). | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------------------------------|---------------------|------|-------|--|
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | The study indicated that the statistical analysis used was based on the dose-response relationship. Therefore, it is inferred from the text that the dose-relatedness/statistical significance of the response was the criteria for a positive response. |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | Cytotoxicity endpoints were defined (as a reduction in the background lawn and/or a reduction in the number of revertant colonies), but the methods of measurements were not fully described or reported. |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Results were reported by exposure group. |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: Araki, A; Kamigaitao, N; Sasaki, T; Matsushima, T (2004). Mutagenicity of carbon tetrachloride and chloroform in Salmonella typhimurium TA98, TA100, TA1535, and TA1537, and Escherichia coli WP2uvrA/pKM101 and WP2/pKM101, using a gas exposure method Environmental and Molecular Mutagenesis, 43(2), 128-133 | | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | | | | | | |
| HERO ID: 194641 | | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | The test substance was identified by CASRN. | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | The source of the test substance was reported, including manufacturer and batch/lot number. | |
| | Metric 3: Test Substance Purity | High | × 1 | 1 | The test substance purity was reported. | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | Negative control was reported. | |
| | Metric 5: Positive Controls | Medium | × 2 | 4 | The authors reported testing positive control substances by the pour plate method, but not the gas-phase exposure method. | |
| | Metric 6: Assay Procedures | High | × 1 | 1 | Study authors described the methods and procedures. | |
| | Metric 7: Standards for Tests | Not Rated | NA | NA | Standards for test provided. The QC part of this test criteria may not be applicable. | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 8: Preparation and Storage of Test Substance | Not Rated | NA | NA | This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage. | |
| | Metric 9: Consistency of Exposure Administration | Medium | × 1 | 2 | Authors reported the details of exposure administration. | |
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | The exposure doses/concentrations or amounts of test substance were reported. | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported. | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The number of exposure groups and dose/concentration spacing were justified by study authors. | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | Study authors reported exposures were conducted in the presence and absence of metabolic activation and the type and source, method of preparation. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | Authors provided descriptive information on the test model. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The authors provided details about the tester strains used in this study. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The authors reported the outcome methodology for the study. | |
| Continued on next page ... | | | | | | |

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

Data Type:
HERO ID: 194641

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

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

| Study Citation: | L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | CCl4 in vitro DNA repair test in E. coli | | | | | |
| HERO ID: | 194717 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as Carbon tetrachloride | |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The source of the test substance was not specifically reported, but it was noted that the chemicals tested were purchased from a commercial source. The product number and batch/lot number were also not reported; however, the material is not expected to vary in composition. The omitted details are unlikely to have a substantial impact on the results. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity and/or grade of the test substance were not reported. It was noted that all chemicals tested were of the highest purity obtainable. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Study authors report using a concurrent negative solvent control; however, the solvent used for carbon tetrachloride was not specified. This limitation is unlikely to have a substantial impact on results. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Several compounds commonly used as positive controls were included in the study and produced positive results. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were partially described, but appear to be appropriate. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported; the solutions were made immediately before the experiment and did not need to be stored. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The highest concentration was reported as well as the dose where no surviving colonies were found and the dose where a statistically significant reduction of the number of colonies of each strain was seen . | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported (1 day) | |
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| Study Citation: | L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | CC14 in vitro DNA repair test in E. coli | | | | | |
| HERO ID: | 194717 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | The number of exposure groups and dose/concentration spacing were justified by study authors (diluted in 7 half log steps or 2-fold dilution steps; but only 3 concentrations were specified (the highest concentration tested, the concentration where no surviving colonies were found, and the concentration where a statistically significant reduction of the number of colonies of each strain was seen) . Though not all exposure concentrations were reported, it is unlikely to have a substantial impact on results. | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | Exposures were conducted in the presence and absence of a metabolic activation system. The source and method of preparation were reported. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test models and source were reported and appropriate for the outcome of interest. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The volume of bacterial mix was reported. One plate per concentration was tested. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently for all three experiments. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each group. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Medium | × 1 | 2 | Statistical methods were described and appropriate for the dataset. It was noted that the confidence interval was determined according to the variance for each strain from a previous experiment; this data was not presented. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The scoring/evaluation criteria was reported (if the number of colonies was < 2 standard deviations of the mean for the strains, the test was considered significant). | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | Cytotoxicity endpoints were not defined. | |
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Study Citation: L. Hellmér, G. Bolcsfoldi (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds Mutation Research, 272(2,2), 145-160
 Data Type: CC14 in vitro DNA repair test in E. coli
 HERO ID: 194717

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|-------------------|---------------------|------|-------|---|
| Metric 25: | Reporting of Data | Low | × 2 | 6 | Data for exposure-related findings were not shown for each study group. |
| Overall Quality Determination [‡] | | High | | 1.6 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 32: **In vitro** evaluation results of Onfelt 1987 for a study on aneuploidy

| Study Citation: | A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Aneuploidy-CCL4 | | | | | |
| HERO ID: | 194719 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance (Carbon tetrachloride) was identified by established nomenclature. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Source for this compound is not clear. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity reported as p.a grade | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Pooled negative controls were used. The test substance was not diluted in a solvent, it is therefore assumed untreated negative controls were used although this was not explicitly stated. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive controls were included but may not be necessary for this study type, the test substance gave a positive response | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Limited, but sufficient methodological details were provided | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not necessary for this study type | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Limited details on test substance preparation were provided. Details did not include mixing, addressing homogeneity or volatility, or storage. | |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Details of exposure administration were inferred from the text and assumed to be consistent across groups | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations (M) were reported however, the study indicated that the concentrations were inferred, or based on water solubility estimates given in the literature (1mg/2000mL). The stock solutions were assumed to be saturated based on this data. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration (3 h for aneuploidy) was clearly reported and appropriate for the outcome of interest. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Only two concentrations were tested, which is lower than the recommended three test groups. | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | V79 Chinese hamster lung cells were an appropriate test model for the outcome of interest. | |
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| Study Citation: | A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154 | | | | | |
|--|---|----------------------------|------|-------|---|--|
| Data Type: | Aneuploidy-CCL4 | | | | | |
| HERO ID: | 194719 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | A single experiment (replicate) per exposure concentration was tested. Single treated cultures would considered acceptable if three concentrations were tested and the recommended number of cells were scored. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome methodology was appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Consistency across study groups was inferred from the text | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | For each concentration approximately 100-200 metaphase cells/slide were evaluated which is lower than the recommended 300 cells. | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The test slides were reported to be coded. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No confounding variables in the test design or procedure were identified. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables in outcomes unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The incidences of aneuploidy from treated samples were compared with controls using a Chi-square test. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | A significant or positive score (increased incidence of aneuploidy) was based on statistical significance. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cell survival was reported as a % of control. Methodological details for determining survival are vague, cells were counted however, use of PI was not explicitly stated. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for all exposure groups was adequately presented. | |
| Overall Quality Determination [‡] | | High → Medium [§] | | 1.4 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "Suggested downgrade to medium for uncertainty about the exposure concentrations and only two exposure groups used in the study."

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154 | | | | | |
| Data Type: Aneuploidy-chloroform | | | | | |
| HERO ID: 194719 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance (Chloroform) was identified by established nomenclature. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Source for this compound is not clear. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity reported as p.a grade |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Pooled negative controls were used. The test substance was not diluted in a solvent, it is therefore assumed untreated negative controls were used although this was not explicitly stated. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive controls were included but may not be necessary for this study type; other test substances gave a positive response. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Limited, but sufficient methodological details were provided |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not necessary for this study type |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Limited details on test substance preparation were provided. Details did not include mixing, addressing homogeneity or volatility, or storage. |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Details of exposure administration were inferred from the text and assumed to be consistent across groups |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations (M) were reported however, the study indicated that the concentrations were inferred, or based on water solubility estimates given in the literature (1ml/200mL). The stock solutions were assumed to be saturated based on this data. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration (3 h for aneuploidy) was clearly reported and appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Three exposure concentrations were tested, some discussion about the chosen concentrations was provided. The spacing appeared to be appropriate based on the study results. |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Not applicable for the study design |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | V79 Chinese hamster lung cells were an appropriate test model for the outcome of interest. |
| Continued on next page ... | | | | | |

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| Study Citation: | A. Onfelt (1987). Spindle disturbances in mammalian cells: III: Toxicity, c-mitosis and aneuploidy with 22 different compounds: Specific and unspecific mechanisms Mutation Research: Environmental Mutagenesis and Related Subjects, 182(3,3), 135-154 | | | | | |
|--|---|----------------------------|------|-------|---|--|
| Data Type: | Aneuploidy-chloroform | | | | | |
| HERO ID: | 194719 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | A single experiment (replicate) per exposure concentration was tested. Single treated cultures is considered acceptable if the recommended number of cells are scored. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome methodology was appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Consistency across study groups was inferred from the text | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | For each concentration approximately 100-200 metaphase cells/slide were evaluated which is lower than the recommended 300 cells. | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The test slides were reported to be coded. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No confounding variables in the test design or procedure were identified. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables in outcomes unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The incidences of aneuploidy from treated samples were compared with controls using a Chi-square test. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | A significant or positive score (increased incidence of aneuploidy) was based on statistical significance. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cell survival was reported as a % of control. Methodological details for determining survival are vague, cells were counted however, use of PI was not explicitly stated. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for all exposure groups was adequately presented. | |
| Overall Quality Determination [‡] | | High → Medium [§] | | 1.4 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

§ Evaluator's explanation for rating change: "Suggested downgrade to medium for uncertainty about the exposure concentrations used in addition to other limited methodological details."

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: I. F. Oruambo, B. L. Van Duuren (1987). Distribution of carbon tetrachloride-metabolite(s) to DNase I-sensitive and -resistant chromatin <i>Cancer Letters</i> , 37(3,3), 311-316 | | | | | |
| Data Type: DNA binding – mouse liver chromatin | | | | | |
| HERO ID: 194721 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (radio-labeled [14C] CCl4) |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported New England Nuclear) |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported. However, the specific activity was reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | The study design investigated binding regions of radiolabeled CCl4 in mouse chromatin; a negative control may not be required for this study. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A concurrent positive control was not used; however, the study design investigated binding regions of radiolabeled CCl4 in mouse chromatin; a positive control may not be required for this study |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Study authors described the methods and procedures; DNA isolation and purification methods were described in previously published studies. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported. Storage of the test substance was not reported; however, because it is a short-term study, this is appropriate. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the treated groups (2-hour and 4-hour groups) |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The exposure concentration of the test substance was reported (2.5 umol [14C]CCl4) |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate for the outcome of interest (2- and 4-hour incubations) |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Only one exposure concentration was tested for 2 exposure durations to measure DNA binding. This dose appeared to be sufficient for assessing the outcome of interest. |
| Metric 13: | Metabolic Activation | Low | × 1 | 3 | Exposures included microsomal protein; however, no descriptive details were reported |
| Domain 4: Test Model | | | | | |
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Study Citation: I. F. Oruambo, B. L. Van Duuren (1987). Distribution of carbon tetrachloride-metabolite(s) to DNase I-sensitive and -resistant chromatin *Cancer Letters*, 37(3,3), 311-316
 Data Type: DNA binding – mouse liver chromatin
 HERO ID: 194721

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 14: Test Model | High | × 2 | 2 | The test model and descriptive information was reported (mouse liver chromatin) |
| | Metric 15: Number per Group | High | × 1 | 1 | Four replicates were included in the study design. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed the intended outcome of interest and was sensitive for that outcome |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for this study design |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information regarding the condition of mice the liver chromatin were derived from was not described, though the characteristics of isolated chromatin was. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis was not conducted. Results present the average and standard deviation of 4 experiments; independent analysis may be conducted. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Data was evaluated as the measure of metabolites bound to DNA in two regions of the chromatin structure. |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | Assay was conducted on chromatin DNA |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data was summarized as means and standard deviations of 4 experiments. Data for each experiment was not reported. |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | M. I. Díaz Gómez, J. A. Castro (1981). Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases Research Communications in Chemical Pathology and Pharmacology, 32(1,1), 147-153 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA Base Interactions with ¹⁴ CCl ₄ | | | | | |
| HERO ID: | 194767 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (radio-labeled [¹⁴ C] CCl ₄) | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | 14CCl ₄ was purchased from the Radiochemical Centre (England). | |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported. However, the specific activity was reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | A simultaneous control not containing the DNA base was included in the study design and processed in the same manner. The control value was subtracted from that of other treatment groups. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were described, but some details were lacking (humidity, pH, volume of saturated solution of each base used, etc.). | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation of the test substance was described. [¹⁴ C]CCl ₄ was added to an ampoule previously sealed under N ₂ with the DNA base saturated in 3 mL of absolute ethanol and 1 mg benzoyl peroxide for 16 hours at 80 deg C. Exposure duration was short (16 hours), therefore details on storage were not required. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | It appears the same methods of exposure were used for each DNA base and control without the base. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentration of [¹⁴ C]CCl ₄ was reported as 25.5E6 dpm/mL. Degradations per minute (dpm) can be converted to mCi and the quantity of [¹⁴ C]CCl ₄ in mmol can be calculated based on the specific activity of 3.81 mCi/mmol. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was 16 hours, which appeared to be appropriate given that DNA binding was observed. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Justification for the concentration of [¹⁴ C]CCl ₄ used was not reported., but appeared to be appropriate given that DNA binding was observed. One concentration tested in a DNA assay is acceptable. | |
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| Study Citation: | M. I. Díaz Gómez, J. A. Castro (1981). Reaction of trichloromethyl free radicals with deoxyribonucleic acid bases Research Communications in Chemical Pathology and Pharmacology, 32(1,1), 147-153 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA Base Interactions with 14CCl4 | | | | | |
| HERO ID: | 194767 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | This metric is not required for this study type. Metabolic activation was not used. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The 4 DNA bases, guanine, adenine, cytosine, and thymine, were used and purchased from Sigma Chemical Co. The animal/cell line of origin for the DNA was not reported, but this is not expected to have substantially impacted results, given that they were obtained from a commercial source. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The number of replicates per DNA base were not reported; however, appears to be a single assay per group. This is considered acceptable for a DNA binding assay. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology and results reported the interaction of trichloromethyl radical with the different DNA bases both by measuring the moles of 14C bound per mol of DNA base (table) and the reaction products (graphs). | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | No inconsistencies were noted and it appears the outcomes were assessed consistently in each DNA base, although it was not specifically stated. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design, as no subjective outcomes were assessed. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial conditions were not reported for each study group, but this is not expected to have substantially impacted results. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on outcome differences unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria of radioactivity was described and results were reported in a table and graphs. Radioactivity was counted by adding water, NCS, PPO, in toluene to the fractions following evaporation. Column chromatographic analysis was performed on reaction products. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Results were provided for each DNA base (less the control value), although the results for the blank control without the DNA base were not reported. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------|---------------------|------|-------|------------------------|
| Overall Quality Determination [‡] | | High | | 1.5 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in <i>Aspergillus nidulans</i> <i>Mutagenesis</i> , 8(4,4), 301-305 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Somatic segregation assay for CCl4 | | | | | |
| HERO ID: | 194776 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (CCl4). | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not reported; however, may have been in a previously published study (Crebelli et al., 1992); it was indicated that CCl4 was re-tested in this study. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity and/or grade of the test substance was not reported; however, may have been reported in a previously published study (Crebelli et al., 1992); it was indicated that CCl4 was re-tested in this study. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using an untreated solvent control (DMSO). | |
| Metric 5: | Positive Controls | Low | × 2 | 6 | A positive control was tested (benomyl at 5 ug/mL) and induced increased numbers of whole chromosome segregants in abnormal colonies (not clear these data were analyzed statistically); in addition, survival for the positive control group was apparently reduced to 19%. | |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | Methods and procedures were not well-described; some aspects of the assay procedures were cited to other publications. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | It can be inferred that the test substance was dissolved in solvent. Storage was not reported. | |
| Metric 9: | Consistency of Exposure Administration | Not Rated | NA | NA | Exposure methods were described in a previously published paper (Crebelli et al., 1992). | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Test concentrations were reported without ambiguity (Table II). | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Not Rated | NA | NA | Assay methods were described in a previously published paper (Crebelli et al., 1992). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number of exposure groups concentration spacing were reported (5 plus controls) and justified by the study authors (based on a previously published study and re-tested in order to obtain more precise dose-response information). | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Not applicable for this study type. | |
| Domain 4: Test Model | | | | | | |
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| Study Citation: | R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in <i>Aspergillus nidulans</i> <i>Mutagenesis</i> , 8(4,4), 301-305 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Somatic segregation assay for CCl4 | | | | | |
| HERO ID: | 194776 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Test Model | Low | × 2 | 6 | The test model was reported with limited descriptive information; test model characteristics may have been better described in a previously published study (Crebelli et al., 1992). | |
| | Metric 15: Number per Group | Not Rated | NA | NA | Assay methods were described in a previously published paper (Crebelli et al., 1992). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodology was described in a previously published study (Crebelli et al., 1992), though limited details appear appropriate for the endpoint of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | The outcome assessment methodology was described in a previously published study (Crebelli et al., 1992), though limited details suggest that outcomes were assessed consistently. | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | The number of colonies scored were reported (range 227 to 671). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on the initial condition for each study group or replicate was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical methods were reported used (chi-square test) and reported in Table II. | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Evaluation criteria were partially reported; statistical analyses and the dose-relatedness of the response were factored into the determination of a positive response. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Toxicity was accounted for and measured as % survival, though methods were not fully described. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for the outcome was presented quantitatively for the outcomes by exposure group. | |
| Overall Quality Determination [‡] | | Medium | | 1.9 | | |
| Extracted | | Yes | | | | |
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Study Citation: R. Benigni, C. Andreoli, L. Conti, P. Tafani, M. Cotta-Ramusino, A. Carere, R. Crebelli (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in *Aspergillus nidulans* *Mutagenesis*, 8(4,4), 301-305
 Data Type: Somatic segregation assay for CCl4
 HERO ID: 194776

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 37: **In vitro** evaluation results of Díaz Gómez et al., 1980 study on DNA binding

| Study Citation: | M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206 | | | | |
|-------------------------------------|--|---------------------|------|-------|---|
| Data Type: | DNA binding of CCl4 metabolites | | | | |
| HERO ID: | 194790 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name and formula. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of the radiolabeled CCl4 (Radiochemical Centre, Amersham) was provided. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | 99% pure as analyzed by glc analysis. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Controls are not needed for DNA binding studies. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Controls are not needed for DNA binding studies. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were partially described and/or cited in another publication but appeared to be appropriate. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation was described, but storage conditions were not indicated. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure appears consistent across groups/replicates. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Specific gravity of radiolabeled compound was provided. In an experiment where cold CCl4 concentration was added to the incubation, concentration was provided in mM. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | 30 minute exposure duration was appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | One experiment with radiolabeled compound; second experiment with cold CCl4 was added. This is adequate for the outcome of interest. |
| Metric 13: | Metabolic Activation | High | × 1 | 1 | Microsomal activation system was used. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | Low | × 2 | 6 | Purified mouse liver DNA was not further described. |
| Metric 15: | Number per Group | High | × 1 | 1 | Triplicate simultaneous experiments were performed. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment methodology was reported (scintillation counting); this method is not very sensitive for the outcome of interest (compared to quantitative analysis for specific adducts). |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was assessed consistently across groups. |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |

Continued on next page ...

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| Study Citation: | M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids <i>Toxicology and Applied Pharmacology</i> , 56(2,2), 199-206 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA binding of CCl4 metabolites | | | | | |
| HERO ID: | 194790 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported across replicates or experiments. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | The footnote to Table 2 indicates that values are significantly higher than controls in Table 1; however no controls are presented in Table 1. No further details were provided. | |
| | Metric 23: Data Interpretation | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data were reported for exposed groups in each experiment; control results were noted in footnote to Table 3 but not reported. However, controls are not required for DNA binding studies. | |
| Overall Quality Determination [‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 38: **In vitro** evaluation results of Doherty et al., 1996 for micronucleus assay

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells <i>Mutagenesis</i> , 11(3,3), 247-274 | | | | | |
| Data Type: Micronucleus assay_CCl4 | | | | | |
| HERO ID: 194804 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance is clearly identified by name (carbon tetrachloride). |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The test substance was not obtained from a manufacturer, but was supplied as a gift (from Dr. R. Crebelli in Rome). Although there did not appear to be analytical verification of the test substance in this study, this study cited publications by Dr. Crebelli (including studies of chlorinated hydrocarbons). |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity/grade of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The report indicates that the study authors used concurrent negative control groups (vehicle was indicated to be culture medium). It appears that all conditions were equal except exposure to the test substance. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Although a concurrent positive control group was not used, the response for CCl4 (and other chemicals) was positive and exposure-related. Therefore, a positive control is not absolutely required. |
| Metric 6: | Assay Procedures | Not Rated | NA | NA | Methods and procedures (including cell density, culture media, incubation temperatures, washing/rinsing methods, and slide preparation) were described. Details of some procedures (e.g., kinetochore labeling) were cited to other publications. Although procedures deviated somewhat from customary practices, they appeared to be applicable to the study type. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation conditions were reported. It was indicated that, owing to insolubility of the test substances (in general), stock solutions were prepared in growth medium at the top concentration to be tested and were placed in an incubator (with shaking) overnight, and then diluted. It was not specified what methods were conducted to minimize loss of the volatile test substance, but it was noted that the exposures were carried out in glass vials, which were assumed to be closed systems for the duration of the exposure; therefore, this is not considered to have substantially impacted the results. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration appeared to be consistent across study groups. |

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Study Citation: A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
 Data Type: Micronucleus assay_CCI4
 HERO ID: 194804

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|------------------------------|--|---------------------|------|-------|---|
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were reported without ambiguity. |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate for the study type. It was noted that, owing to the protocol being used (i.e., use of genetically modified cell lines rather than S9), the exposure duration could be extended to encompass the whole cell cycle (18 hours for AHH-1 cells and 24 hours for MCL-5 and h2E1 cell lines). |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The number of exposure groups (4 plus control) and concentration spacing were considered adequate to address the purpose of the study (e.g., evaluation of exposure-response relationships). Concentrations up to 10 mM were used, which is standard for studies of this type. |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | The study was conducted using metabolically competent cells (rather than an exogenous activation system). The parental cell line used in the study (AHH-1) had only a low level of native CYP1A1 activity; the other two cell lines enabled activation via additional CYP enzymes (CYP2E1 for h2E1 cells, and CYP2E1, 1A2, 2A6, 3A4 and epoxide hydrolase). The study states that genetically modified cells lines such as those used in this study have been shown in other studies to detect metabolites produced from indirect-acting compounds. |
| Domain 4: Test Model | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The cell lines used in the study were obtained from a commercial source (Gentest Corporation); information was provided as to how the MCL-5 and h2E1 strains were derived from the parent (AHH-1 cell line). It was noted as well that the cell lines were cultures for up to 5 weeks to maintain a stable karyotype. The study states that genetically engineered human lymphoblastoid cell lines have been used previously to evaluate clastogenic and aneugenic substances. |
| | Metric 15: Number per Group | High | × 1 | 1 | Duplicate cultures were utilized. The number of replicates was reported and was appropriate for the study type. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed the outcome of interest and appeared to be sensitive to the outcome of interest. In addition to evaluating micronucleus formation, the study went on to characterize the response (via kinetochore labeling to differentiate between aneugenic and clastogenic mechanisms). |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessments were assessed consistently across study groups. |

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| Study Citation: | A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay_CCl4 | | | | | |
| HERO ID: | 194804 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | The study reported adequate sampling for the outcome of interest. It was indicated that 1000 binucleate cells per culture (2000 per exposure level) were examined for the presence of micronuclei (standard for studies of this type). | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | It was reported that slides were coded prior to analysis. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No confounding differences in test design/procedures among study groups were identified. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding differences with respect to outcomes unrelated to exposure were identified. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The study indicates that significant effects (with respect to micronuclei induction) reported in the results and discussion were based on significance in the Chi-squared test at the 99% confidence limit. The results section describes statistically significantly increased micronuclei formation in the various cell lines, largely without reference to specific exposure levels. The accompanying table (Table I-ix for CCl4) and figures do not provide indications of statistical significance; however, raw data are provided, enabling independent statistical analysis. The "lowest significant dose" of induction of kinetochore positive/negative nuclei (from replicate experiments) was provided in an additional table (Table II). | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | The study authors alluded to (but did not explicitly report) the evaluation criteria (i.e., a statistically significantly increase in micronuclei); the evaluation criteria are consistent with studies of this type. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | The study indicates that relative toxicity was evaluated as the proportion of binucleate and mononucleate cells; the proportion of binucleate cells provides an estimate of the nuclear cell division index and this a measure of toxicity. Although the assessment of cytotoxicity was not fully described/accounted for, these omissions are not likely to substantially impact the study results. For example, toxicity at 10 mM CCl4 in all cell lines appeared to be >55% relative to the negative control; however, micronuclei formation was seen at lower exposure concentrations in the absence of substantial (relative) toxicity. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for exposure-related outcomes were reported by exposure group. | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 39: **In vitro** evaluation results of Rocchi et al., 1973 for DNA binding

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425 | | | | | |
| Data Type: DNA binding for CCl4 | | | | | |
| HERO ID: 194878 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The chemical was identified by established nomenclature as carbon tetrachloride (radiolabeled). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity/grade was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Reactions without mouse or rat microsomes served as negative controls. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study type; however, the test substance elicited positive responses. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Procedures and test conditions were described in detail and appropriate for the study design. Other publications were cited for additional details. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Appropriate volumes of undiluted test material were added to the reactions to obtain the desired specific activity/corresponding concentration desired in each reaction. Solutions were appropriately mixed. Test substance storage was not addressed (but not likely to impact the study results). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were consistently administered across study groups. It appears that an additional exposure condition was used for activated mouse microsomes (10.9 umol in addition to 0.218 umol). |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The concentrations tested were reported without ambiguity (e.g., 0.218 umol CCl4). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The duration of exposure was reported (30 minutes) and considered appropriate for the study type. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Although only one dose was used (for all conditions); however, the number of exposure groups was appropriate for the study design. |
| Metric 13: | Metabolic Activation | High | × 1 | 1 | Microsomes were extracted from rats and mice with and without metabolic activation. Details of activation and preparation were provided. |
| Domain 4: Test Model | | | | | |

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| Study Citation: | P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425 | | | | | |
|--|--|----------------------------|------|-------|---|--|
| Data Type: | DNA binding for CCl4 | | | | | |
| HERO ID: | 194878 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model (rat and mouse microsomes) was appropriate for to evaluate the outcome of interest. The extraction of microsomes was cited to another publication. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | It was indicated that extraction of microsomes was performed on the liver of rats and mice in groups of 3. No additional information was provided. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was adequately described and appropriate for the outcome of interest. It was indicated that owing to stringent washing procedures, any binding detected represented true binding (the assessment was sensitive for the outcome of interest). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was evaluated consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. The outcome evaluated was not subjective. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variables in the test design and procedures were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | The data were not amenable to statistical analysis (only 1 test per condition). Data were presented as umol CCl4/mol P DNA. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | Details regarding data interpretation were limited. Without additional discussion, the data provided have less meaning. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | This metric is not applicable to the study design (no cells were used). | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data were provided for each exposure group (rat and liver microsomes, with and without activation, with and without pH enzymes). | |
| Overall Quality Determination [‡] | | High → Medium [§] | | 4.6 | | |
| Extracted | | Yes | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

Table 40: **In vitro** evaluation results of Roldán-Arjona et al., 1991 study on ara mutagenicity assay in *S. typhimurium*

| Study Citation: | T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons <i>Mutagenesis</i> , 6(3,3), 199-205 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | ara mutagenicity assay in <i>S. typhimurium</i> - CCl4 | | | | | |
| HERO ID: | 194881 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as Carbon tetrachloride ("CT") with the correct CASRN and molecular formula. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported (Aldrich and Fluka). The product number and batch/lot number were not reported, but substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity and/or grade of the test substance was reported (provided by the supplier). 99-99.9% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Study authors report using a solvent control (DMSO) | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A concurrent positive control was not used but may not be required for this study. The response of some known carcinogens tested in the study were positive and exhibited a dose-related response for mutations; this indicates that the assay was effective at inducing and identifying a positive mutagenic response. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods and procedures were described; more detailed assay procedures were also described in a previously published studies (Hera and Pueyo, 1986; Roldan-Arjona et al., 1989) | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Test substance preparation was described (dissolved in DMSO). Test substance storage was not reported, but this is appropriate given the study design (single-dose administration). | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across treated and control groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration was reported in Table III without ambiguity | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported (20 minutes) and considered appropriate, as it yielded positive responses from a variety of chemicals tested and was in line with the Ames bacterial reverse mutation assay preincubation method exposure duration (also 20 minutes according to current standards). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number and spacing of exposure concentrations were reported in the results. It was noted that the investigator used a wide range of doses and the compound (negative for mutagenicity) gave a lethal response which indicated that bacteria were adequately exposed | |
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| Study Citation: | T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons <i>Mutagenesis</i> , 6(3,3), 199-205 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | ara mutagenicity assay in <i>S. typhimurium</i> - CCl4 | | | | | |
| HERO ID: | 194881 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (S9 fraction from male rat liver induced with Aroclor-1254). The preparation of the S9 fraction was described in a previous publication (Maron and Ames, 1983). The source, concentration in the final culture and quality control information were not reported. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Not Rated | NA | NA | The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. (<i>S. typhimurium</i> strains BA13 and BAL 13). The source of the bacteria strains were not specified in the report. These strains have been previously described in previously published reports (Ruiz-Rubio et al., 1985; Roldan-Arjona et al., 1989) | |
| | Metric 15: Number per Group | Low | × 1 | 3 | It was reported that at least two plates per dose level were used. This is not considered adequate by current standards for a similar assay (Ames bacterial reverse mutation requires 3 plates per dose level; use of 2 plates per dose level must be scientifically justified). Furthermore, the uncertainty regarding the number of plates per dose level ("at least two") indicates that the data yielded from each test substance and dose level were not obtained by identical procedures. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The AraR bacterial forward mutation assay appeared to be appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | The use of "at least two" plates per dose level indicates that the data yielded from each test substance and dose level were not obtained by identical procedures. It is not clear what the maximum amount of plates per dose level was, so the range of replicates used per dose level is unknown. This is considered to have potentially impacted results. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no confounding variables noted in the study | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding variable unrelated to exposure were identified | |
| Domain 7: Data Presentation and Analysis | | | | | | |

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Study Citation: T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons *Mutagenesis*, 6(3,3), 199-205
 Data Type: ara mutagenicity assay in *S. typhimurium*- CCl4
 HERO ID: 194881

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------------------------------|---------------------|------|-------|--|
| | Metric 22: Data Analysis | Low | × 1 | 3 | A calculation for correlating number of mutations per unit time and per unit dose ("mutagenic potency") with previously established carcinogenic potency was given. However, statistical analysis was not conducted on the data. Although means and standard deviations are provided for each dose level, the number of plates per dose level is uncertain, and therefore independent statistical analysis cannot be conducted. However, statistical analysis is not necessarily required for the Ames bacterial reverse mutation assay, and due to the similarity of the AraR bacterial forward mutation assay, statistical analysis is considered to be not necessarily required for the present data. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The evaluation criteria were reported and appropriate (test compound was considered mutagenic of the number of AraR mutant colonies was at least twice the value of the corresponding solvent control, over at least three dose levels) |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Cytotoxicity endpoints were described (survival) |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for the outcome was presented for the control and treatment groups |
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 41: **In vitro** evaluation results of Roldán-Arjona and Pueyo 1993 for in vitro mutagenicity assay (Ara test) in *S. typhimurium*

| Study Citation: | T. Roldán-Arjona, C. Pueyo (1993). Mutagenic and lethal effects of halogenated methanes in the Ara test of Salmonella typhimurium: Quantitative relationship with chemical reactivity Mutagenesis, 8(2,2), 127-131 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | in vitro mutagenicity assay (Ara test) in <i>S. typhimurium</i> - CCl4 | | | | | |
| HERO ID: | 194882 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (56-23-5); CCl4 | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported. The product number and batch/lot number were not reported; however, the material is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity and/or grade of the test substance was reported (99%). | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Study authors report using a concurrent solvent (DMSO) control. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Positive controls were used (2-aminoanthracene with S9 mixture). | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay methods and procedures were described. The assay procedures were also described in a previously published study (Roldan-Arjona et al., 1989) | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance preparation was described, though storage conditions were not. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across treated and control groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration was reported in the results without ambiguity | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate (3 days). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number and spacing of exposure concentrations were reported in the results; it was noted that the investigator used a wide range of doses for the assays. | |
| Metric 13: | Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (S9 fraction from male liver induced with Aroclor-1254). Volume in the final culture was given. Method of preparation was cited in another publication. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The test models and source were reported and appropriate for the outcome of interest. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 15: Number per Group | High | × 1 | 1 | The number of cells were reported and appropriate. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across the controls and treated groups. |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for this study. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study replicate or group. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical methods were described and appropriate for the dataset. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The evaluation criteria were reported and appropriate. |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cell survival was measured; however, the method of measurement was not explicitly reported. |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data for the outcome was presented for the control and treatment groups; however, data for the positive control (2-AA) was not presented. |
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 42: **In vitro** evaluation results of Galli et al., 1995 study on *S. cerevisiae* AGY3 DEL recombination

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells <i>Carcinogenesis</i> , 16(3,3), 659-663 | | | | | |
| Data Type: <i>S. cerevisiae</i> AGY3 DEL Recombination – CCl4 | | | | | |
| HERO ID: 194889 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | CCl4 was purchased from Aldrich Chemical Company. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Purity was not reported, but because the test substance was obtained from a commercial source, this is not expected to have substantially affected results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Negative control was mentioned in Figures 1a and 1b as a 0.0 mg/mL concentration and in Table II. It was not reported if the control was untreated or solvent. |
| Metric 5: | Positive Controls | High | × 2 | 2 | Several chemicals were also tested in this assay and produced a positive response. Methyl methanesulfonate, ethyl methanesulfonate, and 4-nitroquinoline-1-oxide are all generally recognized as genotoxic and all produced positive responses, indicating the study was capable of detecting a positive response. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods and procedures were described, but some details were lacking (humidity, washing methods, slide preparation, etc.). |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Study used the pre-incubation method under constant shaking. Preparation of the test substance was briefly described. Storage information was not required due to the short exposure duration (16-17 hours). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | It was inferred that exposures were administered consistently across treatment groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Several concentrations were tested and noted on Figures 1a and 1b (appears to be 7 concentrations ranging from 0 to 10 mg/mL). Tabulated results were reported for 8 mg/mL. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Cultures were incubated for 16-17 hours, which appeared to be appropriate for assessing the outcome of interest, given the positive results in several test substances. |
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| Study Citation: | A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells Carcinogenesis, 16(3,3), 659-663 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | S. cerevisiae AGY3 DEL Recombination – CCl4 | | | | | |
| HERO ID: | 194889 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | Justification for the concentrations chosen was not reported. It is unclear how many concentrations were used due to the information being provided in a graph with multiple chemicals included in the same figure (appears to be 7 concentrations ranging from 0 to 10 mg/mL). Toxicity was apparent at the highest dose (% survival). | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to this study type. No metabolic activation was used. Positive responses were observed. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | Saccharomyces cerevisiae strain AGY3 was used for this assay and appears appropriate. Cell cultures were derived in-house and methods were described or referred to those of Gietz et al., 1992 and Rothstein, 1991. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Figure 1a and 1b indicated each experiment was repeated at least 3 times. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The number of recombinants were calculated per 10 ⁴ cells for each colony (DEL events/10 ⁴ survivors in Figure 1a and 1b). Methods for counting the number of recombinants was not reported. 'A minimum increase of 2-fold in a dose-dependent manner has been regarded as evidence for inducibility'. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | No inconsistencies were reported, although the outcome assessment methods were brief. | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | 5 mL cultures containing 3E7 cells/mL were exposed to the test substances, and the number of recombinants were calculated per 10 ⁴ survivors. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design, as no subjective outcomes were assessed. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study replicate or group. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |

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| Study Citation: | A. Galli, R. H. Schiestl (1995). Salmonella test positive and negative carcinogens show different effects on intrachromosomal recombination in G2 cell cycle arrested yeast cells <i>Carcinogenesis</i> , 16(3,3), 659-663 | | | | | |
|--|--|----------------------------|------------------|-------|--|--|
| Data Type: | S. cerevisiae AGY3 DEL Recombination – CCl4 | | | | | |
| HERO ID: | 194889 | | | | | |
| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} | |
| | Metric 22: Data Analysis | Unacceptable | × 1 | 4 | Results were examined based on the fold over controls for the number of DEL recombinants and dose-response was examined. However, no statistical analysis was reported. The fold over control was only reported at 8 mg/mL in Table II. Because results without standard deviations both in the table and graphs, it would not be possible to conduct the statistical analysis/calculations independently. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | 'A minimum increase of 2-fold in a dose-dependent manner has been regarded as evidence for inducibility'. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | % survival was reported in a graph (Figure 1a), although numerical results were only provided at 8 mg/mL. Methods for counting the number of survivors was not reported. | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Figures 1a and 1b show the dose-response and % survival for CCl4, but it is difficult to distinguish exact values due to multiple chemical results being presented on the same graph. Only tabulated results were provided for 8 mg/mL. Quantitative results indicate positive results in the DEL assay for CCl4. | |
| Overall Quality Determination [‡] | | Unacceptable ^{**} | | 1.7 | | |
| Extracted | | Yes | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 43: **In vitro** evaluation results of Galli et al., 1998 study on chromosomal recombination

| Study Citation: | A. Galli, R. H. Schiestl (1998). Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells <i>Mutation Research</i> , 419(1-3,1-3), 53-68 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | Chromosomal recombination for CCl4 | | | | | |
| HERO ID: | 194891 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by name. A CASRN was also provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was identified. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The test substance purity/grade was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Negative controls were run concurrently with each experiment. The negative control was presumably a solvent-only control (indicated in Table 4), but this was not explicitly specified for each independent experiment. The study authors noted that other chemical substances used in the recombination assay arrested in S phase were also considered negative controls (i.e., sodium azide, hydroxylamine hydrochloride, and ethanol). | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls are not required by study type. However, a positive control (cyclophosphamide) was used in the assay that evaluated the role of activation. In addition, the other recombination assays used chemicals that were known carcinogens and/or had tested positive for recombination in previous assays. Test substances used in the assay produced positive responses, indicating that the test system is capable of detecting effects. | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures (all recombination assays) were well-described (e.g., volumes, test conditions, incubation temperature) and appropriate for the study type. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | It was indicated that CCl4 was dissolved in DMSO (with no additional details). Storage conditions were not reported. | |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Exposures were administered consistently across study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations were reported without ambiguity. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration for recombination assays was reported (i.e., 17 hours) and was appropriate for the study type. | |
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| Study Citation: | A. Galli, R. H. Schiestl (1998). Effect of salmonella assay negative and positive carcinogens on intrachromosomal recombination in S-phase arrested yeast cells Mutation Research, 419(1-3,1-3), 53-68 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Chromosomal recombination for CCl4 | | | | | |
| HERO ID: | 194891 | | | | | |
| Domain | Metric | Rating [‡] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | Exposure groups and spacing were appropriate for the outcome of interest (dose-response effects were observed). The justification for the selection of doses was not explicitly specified (presumably based on toxicity and/or previous studies). | |
| | Metric 13: Metabolic Activation | Low | × 1 | 3 | The presence of metabolic activation (30% liver S9) was noted for one of the recombination assays. However, details of preparation were cited to another publication. The species of origin was also not reported. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (<i>Saccharomyces cerevisiae</i> diploid strain RS112) was appropriate for the outcome of interest and described in detail. The strain appears to be laboratory-maintained (used in previous studies). | |
| | Metric 15: Number per Group | High | × 1 | 1 | Reported results (all recombination assays) were from 2 to 3 independent experiments. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate and sensitive for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was consistently assessed across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable for the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable for the study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Confounding variables in test design and procedure were not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | The study considered confounding factors (e.g., evaluated whether an interaction between HU and CCl4 affected results in S-arrested cells). | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Appropriate statistical analysis was performed (Wilcox rank-sum test). Means and standard errors were reported. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Positive results were based on statistical significance or at least a significant two-fold induction with a positive dose-response. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cytotoxicity (cell viability) was reported however, the method used to assess cell viability was not specified. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for all exposure groups is adequately reported. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | P. Foureman, J. M. Mason, R. Valencia, S. Zimmering (1994). Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program Environmental and Molecular Mutagenesis, 23(3,3), 208-227 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | sex linked recessive lethal mutations in drosophila | | | | | |
| HERO ID: | 65173 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance is reported by name, CAS, and structure. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substance source and lot/batch is reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity is reported and adequate. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Concurrent negative control was reported; it is unclear if untreated or vehicle . | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Concurrent positive control is not applicable for this study type. | |
| Metric 6: | Randomized Allocation | Not Rated | NA | NA | This metric is not applicable for Drosophila. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation of the test substance was reported, solutions were renewed at 24 and 48h. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses reported in Table 2 in ppm. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure duration was reported and appropriate for the study type. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Number of exposure groups was selected based on solubility, palatability and toxicity (not further described) for a single dose (each route). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Route and method of exposure were reported and appropriate for the test substance. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Test animals, drosophila, were reported and mating schematic was briefly described and appropriate for the study type. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. | |
| Metric 15: | Number per Group | High | × 1 | 1 | Number of animals in the study were reported and adequate for the outcome. | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was briefly described, previously cited, and appeared adequate for the outcome of interest. | |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was consistent across study groups. | |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | Not applicable to the study type. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable to the study . |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control response was adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Palatability was reportedly included in dose selection, but is not further described. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on health outcomes unrelated to exposure were not reported for each group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was reported and were appropriate for the data. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all exposure groups. |
| Overall Quality Determination [‡] | | High | | 1.5 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 45: **In vitro** evaluation results of Garry et al., 1990 for sister chromatid exchange

| Study Citation: | V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | Sister chromatid exchange for CCl4 | | | | | |
| HERO ID: | 194917 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was specified. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | It was specified that the test substance was spectral grade.; observed effects are likely due to the test substance itself. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | The study reported that both untreated and solvent-only controls were used. It is not clear if dose '0' in Figure 1 pertains to the solvent-only control or untreated control. In addition, the solvent (Pluronic F127, a surfactant) is not routinely used (the difference in SCE response between untreated and solvent-only controls was not reported). | |
| Metric 5: | Positive Controls | Low | × 2 | 6 | The study reported using a positive control (cyclophosphamide) in the presence of activation only (data not shown; presumably a positive response was observed). Although a positive control was not used in the absence of activation, other chemicals used in the study elicited a positive response without activation (indicating that the assay was at least capable of generating a positive response). | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Most of the methods and procedures (e.g., test conditions, culture media and volumes, temperatures) used for the test were reported. Omissions (e.g., details regarding slide preparation) are not likely to substantially impact the study results. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was described (i.e., emulsified in surfactant). Stability of the test substance in the vehicle, which is not routinely used, was not reported. The study indicated that cells were exposed to the test substance in gas-tight glass vials to account for any volatility of the test substance. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses could be estimated from data presented graphically (Figure 1). | |

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial differences with respect to the tissues exposed was not reported. The study only indicated that 5 cc of whole blood was used. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not described clearly (i.e., "analysis of variance procedures and regression analyses" were used). |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | The study authors indicated that the criteria for a positive response was a dose-related increase in SCEs (expressed as SCEs/cell). Based on the information provided, it appears that this was determined by using a trend test . |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Toxicity was assessed, but methods were not well described. |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Data were reported graphically by exposure group for the mean number of SCEs per cell (without a measure of variation). The study authors did not present data in tabular form, and did not present results for numbers of SCEs (overall), or numbers of chromosomes/SCEs per chromosome. |
| Overall Quality Determination [‡] | | Medium | | 2.0 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 46: **In vitro** evaluation results of Garry et al., 1990 for chromosomal aberrations

| Study Citation: | V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Chromosomal aberrations for CCl4 | | | | | |
| HERO ID: | 194917 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was specified. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | It was specified that the test substance was spectral grade.; observed effects are likely due to the test substance itself. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | The study reported that both untreated and solvent-only controls were used. It appears that only untreated controls were conducted concurrently with CCl4 treatments (Table 1 indicated "Solvent control" and "Control" for other compounds, and only "Control" for CCl4). In addition, the solvent (Pluronic F127, a surfactant) is not routinely used. The difference in CA response between untreated and solvent-only controls does not appear to be substantial, but it would instill more confidence if the effect of the surfactant was better described. | |
| Metric 5: | Positive Controls | Low | × 2 | 6 | The study reported using a positive control (cyclophosphamide) in the presence of activation only (one data row shown in Table 1). Although a positive control was not used in the absence of activation, other chemicals used in the study elicited a positive response without activation (indicating that the assay was at least capable of generating a positive response). | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Most of the methods and procedures (e.g., test conditions, culture media and volumes, temperatures) used for the test were reported. Omissions (e.g., details regarding slide preparation) are not likely to substantially impact the study results. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was described (i.e., emulsified in surfactant). Stability of the test substance in the vehicle, which is not routinely used, was not reported. The study indicated that cells were exposed to the test substance in gas-tight glass vials to account for any volatility of the test substance. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity (Table 1). | |
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| Study Citation: | V. F. Garry, R. L. Nelson, J. Griffith, M. Harkins (1990). Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants Teratogenesis, Carcinogenesis, and Mutagenesis, 10(1,1), 21-29 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Chromosomal aberrations for CCl4 | | | | | |
| HERO ID: | 194917 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | Low | × 2 | 6 | The duration of exposure was specified (30 minutes). The duration of exposure was shorter than what is typically used for this study type (3 to 6 hours), the study indicated this duration was chosen because it was relevant to in vivo exposure. | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The study reportedly used 4 doses and included testing up to a cytotoxic dose. The study indicated that doses were selected to delineate dose-response relationships. Based on the data presented in Table 1, it appears that there were 5 doses plus controls and that the highest dose was not analyzable (i.e., toxic) in the presence/absence of activation. | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | The study reported using metabolic activation (S9 rat liver homogenate); the source (a manufacturer) and concentration in final culture were also reported. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (human lymphocytes) was reported and is routinely used for the outcome of interest. The study indicated that blood donors were males aged 25 to 40, without evidence of chronic disease. The study identified several criteria that excluded subjects from the study. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The study reported using duplicate cultures. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology appeared appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently among study groups. | |
| | Metric 18: Sampling Adequacy | Low | × 2 | 6 | Details regarding sampling of outcomes were not fully reported. The study indicated that "one hundred cells per dose with 46 chromosomes were analyzed for aberrations." Typically, about 300 metaphases per dose are sampled (with breakdown by culture specified). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not indicated. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial differences with respect to the tissues exposed was not reported. The study only indicated that 5 cc of whole blood was used. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |

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

Data Type: Chromosomal aberrations for CCl4

HERO ID: 194917

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--|--------------------------------|---------------------|------------------|-------|--|
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not described clearly (i.e., "analysis of variance procedures and regression analyses" were used). |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | The study authors indicated that the criteria for a positive response was a dose-related increase in the mean number of aberrations per 100 cells (presumably based on pairwise comparisons from data in Table 1). However, it appears that data were interpreted in the context of untreated controls rather than solvent controls. |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Toxicity was assessed, but methods were not well described. |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data were reported by exposure group for the mean number of aberrations per 100 cells. The study authors did not present data with respect to number of cells with aberrations, all types of aberrations (e.g., exchanges). number of aberrations including/excluding gaps. |
| Overall Quality Determination [‡] | | Medium | | 1.9 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | R. Braun, J. Schoneich (1975). The influence of ethanol and carbon tetrachloride on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with <i>Salmonella typhimurium</i> Mutation Research, 31(3,3), 191-194 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | S. typh. Mutagenicity, in vitro, spot-test – CCl4 | | | | | |
| HERO ID: | 194934 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Source of CCl4 was not provided. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not provided. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | A concurrent negative control group was not reported. | |
| Metric 5: | Positive Controls | Unacceptable | × 2 | 8 | A concurrent positive control group was not reported and test results were negative. | |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | The assay methods were very briefly described and lacked most details (test conditions, temperature, etc.). | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Test substance was dissolved in 0.9% saline; 0.1 mL spots were applied to center of petri dishes. It is unclear if volatility of the test substance was accounted for; lack of mitigation would substantially affect results. Storage information was not reported but this is unlikely to affect results as the test was performed for a short duration (spot test). | |
| Metric 9: | Consistency of Exposure Administration | Low | × 1 | 3 | Very limited exposure details were provided and it is unclear whether exposures were administered consistently. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations were reported as 10, 20, and 40 mg/mL. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Unacceptable | × 2 | 8 | No information on exposure duration was reported. | |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | Justification for the number of exposure groups and concentrations chosen was not reported. 3 concentrations were used. No information on cytotoxicity was provided so it is unclear whether the high concentration was sufficient as all concentrations yielded negative results. | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Metabolic activation was either not tested or not reported. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | Low | × 2 | 6 | <i>Salmonella typhimurium</i> his G46 and his TA1950 were used. These strains were provided by Professor B.N. Ames (Berkeley). Justification for selection of the strains was not provided. | |
| Metric 15: | Number per Group | Low | × 1 | 3 | Number of replicates per group was not indicated. | |

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| Study Citation: | R. Braun, J. Schoneich (1975). The influence of ethanol and carbon tetrachloride on the mutagenic effectivity of cyclophosphamide in the host-mediated assay with Salmonella typhimurium Mutation Research, 31(3,3), 191-194 | | | | |
|--|--|---------------------|------|-------|--|
| Data Type: | S. typh. Mutagenicity, in vitro, spot-test – CCl4 | | | | |
| HERO ID: | 194934 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Unacceptable | × 2 | 8 | The outcome assessment methodology was not reported |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | The outcome assessment methodology and execution were not reported |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design, as no subjective outcomes were assessed. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study replicate or group. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Statistical analysis was not conducted; however, it is not necessarily required for a bacterial mutation test. |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | Evaluation criteria were not reported. |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Cytotoxicity was not defined or described and it is unclear if cytotoxicity was accounted for in this study. |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Negative results were reported qualitatively, but no data or additional details were provided. Results were not provided for each group. |
| Overall Quality Determination [‡] | | Unacceptable** | | 3.4 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 48: **In vitro** evaluation results of Brennan and Schiestl 1998 for intrachromosomal recombination in yeast

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: R. J. Brennan, R. H. Schiestl (1998). Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in <i>Saccharomyces cerevisiae</i> Mutation Research, 397(2,2), 271-278 Data Type: Intrachromosomal recombination in yeast HERO ID: 194935 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substances (carbon tetrachloride and chloroform) were identified by name and the correct CASRNs. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substances were obtained from a commercial source (Aldrich) |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | It was unclear whether "99%+ pure" referred to CCl ₄ or all test substances listed. However, because chloroform was also obtained from a commercial source, this is not expected to have substantially impacted results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative controls reported; while not explicitly described, no vehicle was used (chemical mixed in medium) so an untreated control is appropriate. |
| Metric 5: | Positive Controls | High | × 2 | 2 | 4-nitroquinoline N-oxide used as positive control |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Test conditions reported in detail including cell concentration, equipment, solution concentrations, incubation period and temperature, and plating. Medium preparation was cited to another publication. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation described (mixed in SC-leu medium) but storage was not reported. This is appropriate given the short duration of study. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure conditions were consistent (cell concentrations, equipment, volumes, etc.) |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses reported as mg/mL in table 1. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Cells incubated for 17 hr. This duration was sufficient to induce recombinations with the positive control. |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | Four doses for CCl ₄ and 5 doses for CHCl ₃ were used. The high dose for both test substances was very toxic (about 1% survival or less) and even the lowest doses reduced survival by ~50%. This is considered to have substantially impacted results. However, it is still considered acceptable because 1) the positive control gave a positive response even with survival at 8-18%, and because both test substance showed a dose-dependent response despite the low survival. |
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| Study Citation: | R. J. Brennan, R. H. Schiestl (1998). Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals in <i>Saccharomyces cerevisiae</i> Mutation Research, 397(2,2), 271-278 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Intrachromosomal recombination in yeast | | | | | |
| HERO ID: | 194935 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | Positive responses observed without metabolic activation | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | Test model was <i>Saccharomyces cerevisiae</i> and strain genotype was described in detail. Standard species for recombination assays. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | Table 1 reports results from "at least two" replicates. Two replicates is considered somewhat lacking, and the vague "at least two" phrase adds uncertainty to this metric. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment cited to another publication with no additional details | |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment cited to another publication with no additional details | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Outcome assessment cited to another publication with no additional details | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | No information on blinding was reported. Outcome assessment cited to another publication with no additional details | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial batch/lot number of organisms was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not conducted. Means and standard deviations were reported, but sample sizes ("at least two" replicates) were unclear, so it is not possible to conduct independent statistical analysis. However, this is still considered acceptable, as statistical analysis is not necessarily required for recombination assay (2-fold increase considered positive). | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria reported (2-fold increase considered positive) and cited to other publications. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Survival evaluation methods were cited to another publication. Cell survival was reported for all doses and control | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Survival and DEL recombination frequencies reported for all doses and assay conditions; means and SDs reported; results for negative and positive controls reported. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | V. V. Khudoley, I. Mizgirev, G. B. Pliss (1987). The study of mutagenic activity of carcinogens and other chemical agents with <i>Salmonella typhimurium</i> assays: Testing of 126 compounds <i>Archiv für Geschwulstforschung</i> , 57(6,6), 453-462 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: | 194949 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride with the correct CASRN. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The commercial source of CCl4 was not reported. A subset of the 126 test substances were reported to have been synthesized at the home institution of the authors, so it can be assumed that the CCl4 was obtained from an unidentified commercial source. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | It was reported that the “majority” of the 126 test substances were “chemically pure”. The purity of CCl4 was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Solvent controls were included concurrently in study design. | |
| Metric 5: | Positive Controls | Low | × 2 | 6 | Appropriate concurrent positive control test substances were included for each test condition with and without S9 activation. Positive control data were not reported. | |
| Metric 6: | Assay Procedures | Not Rated | NA | NA | Assay methods and procedures were cited to other publications. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Not Rated | NA | NA | Assay methods were cited to other publications. | |
| Metric 9: | Consistency of Exposure Administration | Not Rated | NA | NA | Assay methods were cited to other publications. | |
| Metric 10: | Reporting of Doses/Concentrations | Not Rated | NA | NA | Assay methods were cited to other publications. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Not Rated | NA | NA | The assay procedures were described as “routine protocol” and cited in other references. | |
| Metric 12: | Exposure Route and Method | Not Rated | NA | NA | The number of exposure groups and dose spacing were not reported. The assay procedures were described as “routine protocol” and cited in other references. | |
| Metric 13: | Metabolic Activation | Medium | × 1 | 2 | The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. | |
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| Study Citation: | V. V. Khudoley, I. Mizgireuv, G. B. Pliss (1987). The study of mutagenic activity of carcinogens and other chemical agents with <i>Salmonella typhimurium</i> assays: Testing of 126 compounds <i>Archiv für Geschwulstforschung</i> , 57(6,6), 453-462 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CC14 | | | | | |
| HERO ID: | 194949 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Not Rated | NA | NA | The number of plates per treatment group was not reported. The assay procedures were described as "routine protocol" and cited in other references. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology is appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this endpoint. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Number of colonies is an objective outcome and blinding assessors is not necessary. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study replicate or group. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Medium | × 1 | 2 | The data were statistically analyzed, but the statistical test was not reported. A positive result was defined as a dose-dependent response at least 2x background mutation rates, which is appropriate for this study design. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | No cytotoxicity assay was included for the bacterial mutagenicity assay; however, this is unlikely to have a substantial impact on the study results. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | All data are adequately reported. | |
| Overall Quality Determination [‡] | | Medium | | 1.7 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234 | | | | | |
| Data Type: Mitochondrial and nuclear DNA binding for CCl4 | | | | | |
| HERO ID: 194952 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as 14C-labelled carbon tetrachloride (CCl4). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was identified (Amersham). The product number and batch/lot number were not reported; however the material is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The radiochemical purity of the test substance was reported (>99%). |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | The use of a negative control group is strictly required; measurement of the radiolabeled test compound is the outcome. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control was not required by study type; however, treatment-related results were observed (indicating that the assay was effective). |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | Assay methods and procedures were partially described. Mitochondria preparation was cited to another publication (Brabec et al., 1975). Details of mitochondrial DNA preparation were omitted. Information about the assay using calf thymus nuclear DNA were not provided. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Details regarding test substance preparation were limited. Storage was not reported (but not expected to impact study results). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | It is inferred that exposures were administered consistently across study groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The dose was reported without ambiguity (30 mM CCl4 containing 25 uCi 14C-labelled CCl4). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported (30 minutes) and appeared adequate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | One concentration was used; however, this concentration was sufficient to detect DNA binding. |
| Metric 13: | Metabolic Activation | Low | × 1 | 3 | The presence of a metabolic activation system was reported (post-mitochondrial fraction), but was not validated. It is not clear if the PMS is an adequate activation condition for calf thymus DNA (especially in the absence of a positive response). |
| Domain 4: Test Model | | | | | |

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| Study Citation: | G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Mitochondrial and nuclear DNA binding for CCl4 | | | | | |
| HERO ID: | 194952 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Test Model | Low | × 2 | 6 | The test models were reported with limited (mitochondrial suspension) to no (calf thymus DNA) additional information. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The study indicated that results for mitochondrial DNA were based on 4 experiments and results using calf thymus DNA were based on 3 experiments. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the endpoint of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on initial conditions for each study group was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Statistical analysis was not conducted. Data were provided as means +/- standard deviations for a specified number of experiments (n = 3 or 4). | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | The criteria for a positive response was not explicitly specified (other than the detection of radiolabel in isolated DNA). | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | This metric is not applicable to the study type (no cells were used). | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data were reported by exposure group. | |
| Overall Quality Determination [‡] | | Medium | | 1.7 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

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

| Study Citation: | G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | DNA and nuclear protein binding | | | | | |
| HERO ID: | 194983 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as CCL4, CASRN provided. Radiolabeled CCL4 also used. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Commercial sources were reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Unlabeled CCL4 reported to be "low sulfur quality", - labeled CCL4 purity 99% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Not applicable for the study design (DNA binding/adduct assays) | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Not applicable for the study design | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay procedures were cited to another publication but with some details briefly described. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Details of exposure were described. Storage of radiolabeled CCL4 was reported and stability was tested/confirmed | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were consistent for each test condition and performed under an N2 atmosphere. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The exposure concentration was reported (0.2mM radiolabeled CCL4) | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The reaction duration (1hr) was reported. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Only a single dose tested, however, this was appropriate for the study design and the outcome of interest. | |
| Metric 13: | Metabolic Activation | High | × 1 | 1 | Experiments were performed in the presence and absence of an NADPH generating system. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | Nuclear DNA and protein preparations from livers of three species (mouse, rat, and hamster) | |
| Metric 15: | Number per Group | High | × 1 | 1 | Experiments in each species were performed in triplicate. | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Assessment methods were cited to another publication, but details were briefly described and the methods appeared appropriate. | |

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| Study Citation: | G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | DNA and nuclear protein binding | | | | | |
| HERO ID: | 194983 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Outcome assessment appeared to be consistent across species. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Confounding variables in test design and procedures were not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | Purity of the nuclear preparations were tested and confirmed | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis to evaluate differences between species and results in the presence and absence of NADPH was performed and appropriate (student T-test). Data were presented as Means with SD | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Data interpretation was appropriate for the outcome of interest. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | Not applicable (no cells) | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | All experimental data was adequately reported | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 52: **In vitro** evaluation results of Coutino 1979 for chromosomal abnormalities

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136 | | | | | |
| Data Type: CA-CCL4 | | | | | |
| HERO ID: 195013 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified by name as CCL4, CASRN not provided. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Test substance source not reported |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity not reported |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent non-solvent (buffer) controls were used as comparators. DMSO was included in the test, but as one of the test substances not as a control. The text suggests compounds were added undiluted or in PBS, it is therefore assumed no solvents were used. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | The study did not specify use of a positive control, however a positive responses were indicated/ reported in the text for several compounds.. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | The Assay methods were sufficiently described and were appropriate for the outcome of interest. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Limited details of test substance preparation (added with PBS or alone) and no details of test substance storage were reported. Test substance was noted as being volatile, however, test procedures do not indicate any measures were taken to account for volatility during exposure. |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Consistency across groups is inferred from the text. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The study indicates that logarithmic or geometric progressions of concentration were evaluated, however only the dose (5ul/mL) producing the highest frequency of anomalies are reported. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration (24hrs) was reported and appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The study indicates that logarithmic or geometric progressions of concentration were evaluated, however the specific number of doses tested was not reported. No further justification of doses were provided. |
| Metric 13: | Metabolic Activation | Low | × 1 | 3 | Metabolic activation was not included; metabolites were not directly tested, however a positive response was observed. |
| Domain 4: Test Model | | | | | |

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| Study Citation: | R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | CA-CCL4 | | | | | |
| HERO ID: | 195013 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (CHO cells) is appropriate for the outcome of interest. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The study indicates there were two dishes/dose however, the study does not indicate these were duplicates, but rather the number of dishes required to obtain the number of cells needed for the outcome assessment. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment methodology was described in limited detail (e.g., cells were analyzed). No additional information was provided. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Details of outcome assessment were limited, although it is assumed the the controls and samples were consistently evaluated. | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | The number of cells analyzed per group was appropriate (500 anaphases) | |
| | Metric 19: Blinding of Assessors | Low | × 1 | 3 | Use of blinding or coded cells were not reported and should be included in this type of study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variables in the test design or procedure were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Confounding variables not related to the outcome of exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Statistical analysis was not included (single dose tested) | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | Details of scoring or evaluation criteria were not described. The text indicates there is a "great increase" without indicating what the threshold is for an increase is. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cytotoxicity was measured indirectly by means of the mitotic index, but additional details of the procedure/assessment were not reported. | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | The text indicated that no compounds had cytotoxic effects at the concentrations producing anomalies, but the data were not provided. Experimental Data were reported as ratios to control. Percentages or incidence data for the exposure group were not reported. | |
| Overall Quality Determination [‡] | | Medium | | 2.0 | | |
| Extracted | | Yes | | | | |
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Study Citation: R. R. Coutino (1979). Analysis of anaphase in cell culture: An adequate test system for the distinction between compounds which selectively alter the chromosome structure or the mitotic apparatus Environmental Health Perspectives, 31 131-136
 Data Type: CA-CCL4
 HERO ID: 195013

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: G. Gualandi (1984). Genotoxicity of the free-radical producers CCl ₄ and lipoperoxide in <i>Aspergillus nidulans</i> Mutation Research, 136(2,2), 109-114 | | | | | |
| Data Type: Somatic segregation for CCl ₄ and chloroform | | | | | |
| HERO ID: 195130 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were clearly identified as carbon tetrachloride (CCl ₄) and chloroform (CHCl ₃). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substances was reported (Merck). |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The test substances were reportedly analytical grade. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | The study authors reported using a concurrent negative control group (presumably untreated). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control was not tested; however, treatment-related positive responses were observed demonstrating that the test is capable of detecting a positive response. |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | Methods and procedures were reported in limited detail. Media preparation was cited to a previous publication (Gualandi and Morpurgo, 1983). |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Storage was not reported (but was not expected to impact the study results). However, there were substantial deficiencies regarding test substance preparation. It appears that test chemicals were added to molten agar. The study indicates that the test substances were not soluble in aqueous media. |
| Metric 9: | Consistency of Exposure Administration | Low | × 1 | 3 | Exposures appeared to be administered consistently across study groups; however, there was not enough information provided to determine if consistent volumes were used. The study also indicated that CCl ₄ decays in the plate (i.e., no control for evaporation). |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration used in this assay was reported without ambiguity (0.5% v/v). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Low | × 2 | 6 | The exposure duration of the plate-mediated somatic segregation assay was not clearly reported (2 to 3 days). |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | Based on information presented in the results, it appears that the study authors intended to use more than one concentration; however, since the test substances were not soluble in aqueous media, only a narrow range of concentrations could be tested. Only one concentration was tested, and toxicity at this dose was >70% for both test substances. |
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| Study Citation: | G. Gualandi (1984). Genotoxicity of the free-radical producers CCl ₄ and lipoperoxide in <i>Aspergillus nidulans</i> Mutation Research, 136(2,2), 109-114 | | | | |
|--|--|---------------------|------|-------|--|
| Data Type: | Somatic segregation for CCl ₄ and chloroform | | | | |
| HERO ID: | 195130 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to the study type (however, fungi possess a cytochrome P-450-dependent monooxygenase system). |
| Domain 4: Test Model | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model was reported along with limited (mainly genotypic) information. Some information pertaining to the diploid strain was cited to another publication (Gualandi and Morpurgo 1983). |
| | Metric 15: Number per Group | High | × 1 | 1 | The study indicated that there were 7 replicates for CCl ₄ and 4 replicates for CHCl ₃ . |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodology was reported in limited detail. It was indicated that the assay was sensitive for the outcome of interest. Cross-overs and deletions were indistinguishable for the test. |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | There was incomplete reporting of details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results. |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on the initial conditions for each study group or replicate was not reported. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on disproportionate outcomes unrelated to exposure were not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | No statistical analysis was conducted; however, means SEMs, and numbers of replicates were provided for independent analyses. |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Evaluation criteria were not described. Based on information presented in the results and discussion, fold-changes over controls were considered as a factor for determining a positive response (e.g., a two- to three-fold increase was considered weakly positive). |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | The study authors provided data as % survival; however, cytotoxicity methods were not described. |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for the outcome was presented quantitatively for the outcome by exposure group. |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.0 | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 54: **In vitro** evaluation results of Gualandi 1984 for gene mutations

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in <i>Aspergillus nidulans</i> Mutation Research, 136(2,2), 109-114 | | | | | |
| Data Type: Gene mutation CCl4 and chloroform | | | | | |
| HERO ID: 195130 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were clearly identified as carbon tetrachloride (CCl4) and chloroform (CHCl3). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substances was reported (Merck). |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The test substances were reportedly analytical grade. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | The study authors reported using a concurrent negative control group (presumably untreated). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control was not tested; however, (weakly) positive responses were observed demonstrating that the test is capable of detecting a positive response. |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | Methods and procedures were reported in limited detail. Media preparation was described in a previous study (Gualandi and Morpurgo, 1983) and the growth-mediated assay cited Bignami et al., (1981). |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Storage was not reported (but was not expected to impact the study results). However, there were substantial deficiencies regarding test substance preparation. It appears that test chemicals were added to molten agar. The study indicates that the test substances were not soluble in aqueous media. |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Exposures appeared to be administered consistently across study groups; however, there was not enough information provided to determine if consistent volumes were used. The study also indicated that CCl4 decays in the plate (i.e., no control for evaporation). |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration used in this assay was reported without ambiguity (0.5% v/v). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Low | × 2 | 6 | Exposure duration were not clearly reported (i.e., 4 to 5 days for the growth-mediated assay and/or until sporulation). |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | Based on information presented in the results, it appears that the study authors intended to use more than one concentration; however, since the test substances were not soluble in aqueous media, only a narrow range of concentrations could be tested. Only one concentration was tested, and toxicity at this dose was approximately 70% for both test substances. |

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| Study Citation: | G. Gualandi (1984). Genotoxicity of the free-radical producers CCl4 and lipoperoxide in <i>Aspergillus nidulans</i> Mutation Research, 136(2,2), 109-114 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Gene mutation CCl4 and chloroform | | | | | |
| HERO ID: | 195130 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to the study type (however, fungi possess a cytochrome P-450-dependent monooxygenase system). | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model was reported along with limited (mainly genotypic) information. Additional information pertaining to the haploid strain was cited to another publication (Lilly 1965). | |
| | Metric 15: Number per Group | High | × 1 | 1 | The study indicated that there were 2 to 3 replicates for CCl4 and CHCl3. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment methodology was reported in limited detail. It was indicated that the assay was not very sensitive for the outcome of interest (especially the plate incorporation assay). | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | There was incomplete reporting of details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on the initial conditions for each study group or replicate was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on disproportionate outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | No statistical analysis was conducted; although means and SEMs were provided, independent analyses could not be performed without information on the specific number of replicates (2 or 3). However, for mutation assays, fold-changes rather than statistical analyses are often used to analyze the study results. | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Evaluation criteria were not described. Based on information presented in the results and discussion, fold-changes over controls were considered as a factor for determining a positive response (e.g., a two- to three-fold increase was considered weakly positive). | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | The study authors provided data as % survival; however, cytotoxicity methods were not described. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data for the outcome was presented quantitatively for the outcomes by exposure group. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.0 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 55: **In vitro** evaluation results of Whittaker et al., 1989 for chromosome loss in yeast

| Study Citation: | S. G. Whittaker, F. K. Zimmermann, B. Dicus, W. W. Piegorsch, S. Fogel, M. A. Resnick (1989). Detection of induced mitotic chromosome loss in <i>Saccharomyces cerevisiae</i> --an interlaboratory study <i>Mutation Research</i> , 224(1,1), 31-76 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Chromosome loss - Yeast | | | | | |
| HERO ID: | 198010 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as carbon tetrachloride; the CASRN was provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial supplier (Radian Corp) was reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity 99% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Untreated and solvent (ethanol) controls were included. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Appropriate positive controls (methyl benzimidazol-2-yl-carbamate) or propionitrile were used and responded as expected. | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were described in detail and were appropriate for the outcome of interest. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance preparations (dilutions from stock) were adequately described and were performed just prior to addition to the cultures. Storage (of stock solutions) was not described. Reactions were performed in sealed containers to account for potential volatilization. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure were reported and methods were consistent across groups | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Test concentrations were clearly reported in mg/mL. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration for each type of test was clearly reported. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure groups and spacing were determined based on a pre-test to identify inhibiting concentrations. Up to 10 doses were tested (minimum 4) in each experiment | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Not applicable for test system (yeast) | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The test model (diploid yeast strain D6.1M) was reported and appropriate | |
| Metric 15: | Number per Group | High | × 1 | 1 | Experiments were performed in triplicate, and some were performed by two independent laboratories. | |
| Domain 5: Outcome Assessment | | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was clearly detailed and appropriate for the outcome of interest. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was consistent between treatment groups and controls. |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable to study type (colonies counted) |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The chemicals tested were coded prior to the start of the experiments. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions (e.g., batch/lot number) per group were not reported. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The methods of statistical analysis were reported. Justification for the methods were provided. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The study authors clearly described evaluation criteria. |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Pre-tests were done to determine the effect of concentrations on cell titers. Cell viability was calculated from the number of colonies on synthetic complete media. Viable titers were reported concurrently with each experiment. |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data for all tests and exposure groups were reported without a measure of variability across replicates |
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 56: **In vitro** evaluation results of Barber et al., 1981 for bacterial reverse mutation

| Study Citation: | E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: | 200219 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. A structure was also provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported (Eastman Organic Chemicals). A batch/lot number was not reported, but the chemical substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of CCl4 as per GLC was 98.5%. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative controls consisted of plates in a closed system with no added test or positive control chemical. With the exception of not adding chemical to the system, untreated controls were treated the same as treatment groups. Negative controls were used for each strain, with and without metabolic activation. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Positive controls were used. It is noted that positive control substances were not volatile, and were (therefore) not subjected to a closed test system. 2-Aminoanthracene was the positive control with activation (all strains). Without activation, ICR-191 was used for <i>S. typhimurium</i> TA 98, methyl-N-nitro-N'-nitroguanidine was used for strains TA 100 and TA 1535, 9-aminoacridine was used for TA 1537, and picrolonic acid was used for TA 1538. Positive controls yielded positive responses. | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | In this study, a modified plate-incorporation test was conducted using a chemically inert, closed-system protocol. Assay methods were described in detail, including the system used and how the addition of CCl4 was handled. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Owing to the volatility of the test substance, doses were confirmed. Plates containing only distilled water were included in the closed system for GLC analysis of aqueous CCl4 concentrations at the end of the 48-hour incubation period. Samples of the vapor were also taken from the closed system containers at the end of the period and analyzed by GLC. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity. Measured CCl4 concentrations were 0, 4.7, 5.7, 10.2, 12.3, and/or 18.4 μmoles/plate. | |

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| Study Citation: | E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: | 200219 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate. Plates were exposed for 48 hours at 37C. The study generated conditions that permitted the tester strains to be exposed to CCl4 as a vapor for the entirety of the 48-hour exposure period (without loss due to volatility). | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | The number of groups was adequate for the study type (at least 4 exposure concentrations plus controls); however, the range of doses was lacking as it covered less than a full order of magnitude. Results were negative at all doses; although the text indicates that the test substance was tested at concentrations sufficient to produce observable toxicity to the tester strains, toxicity is not indicated for CCl4 in Table 4. | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Aroclor-induced rat liver S9 was used. The source was reported (a manufacturer). Details regarding composition were not provided. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. | |
| | Metric 15: Number per Group | High | × 1 | 1 | Table 6 suggests that 5 replicates were used per group. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology is appropriate for the outcome of interest. The number of revertant colonies/plate was counted after 48 hours incubation. Revertant colonies were counted using a colony counter. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this endpoint. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No differences among treatment group parameters were identified. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Increased revertants/plate compared to controls was evaluated using statistical analysis (Student's t-test). Statistics were used to determine the minimum vapor concentration that significantly increased the number of revertant colonies. | |
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Study Citation: E. D. Barber, W. H. Donish, K. R. Mueller (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames salmonella/microsome assay Mutation Research: Genetic Toxicology, 90(1,1), 31-48
 Data Type: Bacterial reverse mutation for CC14
 HERO ID: 200219

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------------------------------|---------------------|------|-------|--|
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria (number of colonies) were reported. The criteria for a positive result was increased revertants/plate compared to controls (analyzed statistically). |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Cytotoxicity was described as absence of a background lawn. Further details were not provided. |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Average spontaneous reversion rates from negative controls were reported (and were reportedly in agreement with those found by an interlaboratory survey by de Serres and Shelby [1979] and those presented by Ames [1975]). Raw data (i.e., individual plate counts) were not provided. Negative data were reported qualitatively (i.e., for S. typhmuri strains TA 1537 and TA 1538). Standard deviations for mean numbers of revertants/plate (except positive and negative controls) were not reported. No historical control data was provided. |
| Overall Quality Determination [‡] | | High | | 1.2 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 57: **In vitro** evaluation results of Crebelli et al., 1988 for *Aspergillus* mitotic segregation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: R. Crebelli, R. Benigni, J. Franekic, G. Conti, L. Conti, A. Carere (1988). Induction of chromosome malsegregation by halogenated organic solvents in <i>Aspergillus nidulans</i> : Unspecific or specific mechanism? <i>Mutation Research</i> , 201(2,2), 401-411 | | | | | |
| Data Type: <i>Aspergillus</i> mitotic segregation_ CCl4 | | | | | |
| HERO ID: 200282 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance is clearly identified by name (carbon tetrachloride) and CASRN (56-23-5). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported (purchased from Carlo Erba, Milan). Although a batch/lot number was not provided, the substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of the test substance was reported (>99.5%); any observed effects are highly likely caused by the test substance itself. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported the use of negative controls; all conditions (except for addition of the test substance) appeared to be equal. |
| Metric 5: | Positive Controls | Medium | × 2 | 4 | A positive control (benomyl) was reported. There were uncertainties associated with the use of this control group. Data for the positive control were shown in Table 2 only (data for CCl4 in Table 1); Table 2 references to historical control values for the positive control whereas the methods indicate the chemical was used in the study (not entirely clear if the control was concurrent, and no statistics were applied to these data). These uncertainties are not expected to substantially affect the study results. |
| Metric 6: | Assay Procedures | Not Rated | NA | NA | Methods and procedures were partially described and/or attributed to other cited publications (e.g., classification of yellow segregants). The procedures appear to be applicable to the study type, and omissions (e.g., cell density) are unlikely to substantially impact the study results. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | The metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Minimal details regarding test substance storage and/or preparation were reported. The study indicates that conidia were treated with the test substance in sealed capped tubes. The lack of additional details is not expected to substantially impact the study results. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were reported without ambiguity. |
| Continued on next page ... | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration appeared to be appropriate for the study type. The study indicated that this protocol is routinely used. Pre-germinating conidia were treated the test substance until the emergence of the germ tube (approximately 3 hours). |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | The number of exposure groups (5 + control) and concentration spacing were justified by the study authors and appeared to be adequate to address the purpose of the study. The study indicated that a wide range of concentrations was applied to determine the lowest and highest effective doses as well as the lowest concentration arresting conidial germination or inducing a lethal hit per cell. |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | The metric is not applicable to this study type. |
| Domain 4: Test Model | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The strain was generated (and was presumably maintained) by the laboratory that conducted the study. Limited descriptive information about the strain (<i>A. nidulans</i> diploid strain P1) was provided (i.e., genetic information). The study indicates that the test model organism is a common choice for the detection of chemically induced chromosome missegregation. |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The study does not make reference to replicates; there may have been only one per exposure group. However, this limitation is unlikely to substantially impact the study results. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment addressed the intended outcome of interest (i.e., the frequency of mitotic segregants). |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment protocol was applied consistently across study groups. |
| | Metric 18: Sampling Adequacy | Low | × 2 | 6 | Uncertainties were identified with respect to the outcome of interest. A large number of colonies were scored. However, the number of colonies scored ranged from 2371 in controls to only 182 in the highest exposure group (presumably due to decreased germination at higher concentrations). |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | The metric is not applicable to this study type. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No confounding differences in test design/procedures among study groups were identified. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding differences with respect to outcomes unrelated to exposure were identified. |
| Domain 7: Data Presentation and Analysis | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---------------------|---------------------|------|-------|--|
| Metric 22: | Data Analysis | High | × 1 | 1 | Statistical methods were applied to the data, and appeared to be appropriate for the study type. Statistical significance was clearly reported in the data table (p< 0.05 or P<0.001 based on chi-square test). Raw data were provided, enabling independent statistical analysis. |
| Metric 23: | Data Interpretation | High | × 2 | 2 | The study indicated that "positive" mitotic segregants were detected as homo- or hemizygous yellow sectors or patches in heterozygous pale green colonies. Segregants were further classified as mitotic crossovers or non-disjunctional diploids or haploids. These evaluation criteria appear to be consistent with routine methods for this study type. |
| Metric 24: | Cytotoxicity Data | Medium | × 1 | 2 | The study identified the lowest exposure concentration that arrested conidial germination for other test substance, but this was not seen at the doses tested for CCl4. The study authors suggested that increased missegregation was induced at concentrations that affected cell division, but did not block division (i.e., at doses up until arrest was observed). |
| Metric 25: | Reporting of Data | High | × 2 | 2 | Data for exposure-related outcomes were reported by exposure group. |
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 58: **In vitro** evaluation results of Garrett and Lewtas 1983 for inhibition of DNA and protein synthesis

| Study Citation: | N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465 | | | | |
|--|---|---------------------|------|-------|--|
| Data Type: | Inhibition of DNA and protein synthesis for CCl4 | | | | |
| HERO ID: | 626038 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The test substance was commercially sourced. Although the name of the manufacturer was not reported, this omission is not likely to substantially impact the study results. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The specific purity of the test substance was not reported, but it was noted that every chemical tested was "reagent grade and the highest purity commercially available." |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Negative solvent controls were included. It is noted that water insoluble compounds were dissolved "with small amounts of acetone, ethanol, or DMSO;" it was not specified which solvent was used for each test substance. However, the study indicated that appropriate solvent controls were used. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods presented in the study report were described adequately; however, methods associated with cytological and ATP analyses were cited to another publication. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | It was not described how volatile test substances were handled. This is considered to have substantially impacted results. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The methods and Table 1 indicate that the test substance was evaluated at 1000 ug/mL. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and considered appropriate for the study type (20 hr). |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | The study report suggests that one dose was tested (prescreening) rather than at least two as recommended for similar study types. |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | Chinese hamster ovary (CHO) cells were utilized for this study. The identity, source, and culture methods for the CHO cells were reported. This cell line is routinely used for genotoxicity endpoints. |

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| Study Citation: | N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Inhibition of DNA and protein synthesis for CCl4 | | | | | |
| HERO ID: | 626038 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The methods indicate that each experimental condition was conducted with n = 3 technical replicates, with n = 5 replicates for controls.; each experiment was conducted twice. Based on data presented in Table 1, it appears that at least 6 replicates were used for CCl4. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology is appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No differences among treatment group parameters were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding variables were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Unacceptable | × 1 | 4 | No statistical analysis was performed, and raw data were not provided to enable independent statistical analysis. The data shown for CCl4 in Table 1 (DNA synthesis) are shown as the percentage of control. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | The criteria for a positive response was not reported. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Cytotoxicity endpoints were defined in the study report, and methods used for assessing cytotoxicity were adequately described (i.e., trypan dye exclusion). | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Data were reported for the 1000 ug/mL group only (Table 1); data were expressed as the percentage of the control (i.e., control data were not shown). | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.6 | | |
| Extracted | | No | | | | |
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Study Citation: N. E. Garrett, J. Lewtas (1983). Cellular toxicity in Chinese hamster ovary cell cultures: I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants Environmental Research, 32(2,2), 455-465
 Data Type: Inhibition of DNA and protein synthesis for CCl4
 HERO ID: 626038

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 59: **In vitro** evaluation results of Imperial Chemical Industries Ltd. 1976 for genotoxicity-bacterial reverse mutation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: Imperial Chemical Industries Ltd. (1976). Mutagenicity testing with salmonella typhimurium strains on plates, of gases, liquids and solids for Imperial Chemical Industries Limited with attachments | | | | | |
| Data Type: Genotoxicity-bacterial reverse mutation | | | | | |
| HERO ID: 4215890 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | Medium | × 2 | 4 | Test substance identified; CASRN not reported. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | Test manufacturer of the substance was reported, but batch/lot number was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity and grade of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Authors reported the use of negative controls. |
| Metric 5: | Positive Controls | High | × 2 | 2 | Authors reported use of positive controls. |
| Metric 6: | Assay Procedures | Low | × 1 | 3 | Assay procedure was described with limited assay details. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | The QC part of this test criteria may not be applicable. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Not Rated | NA | NA | This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage. |
| Metric 9: | Consistency of Exposure Administration | Low | × 1 | 3 | Although exposure administration information was provided it is incomplete making it less consistent. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Authors reported three doses/concentrations of the test chemical. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Low | × 2 | 6 | The exposure duration reported was lower than standard assays. |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | Although the number of exposure groups was sufficient, the higher and highest exposure concentrations were highly toxic. |
| Metric 13: | Metabolic Activation | Low | × 1 | 3 | The authors reported the use of metabolic activation, however, the methods lack the details of the protocol. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | Medium | × 2 | 4 | The authors reported the strain types, their properties and the description of the test model, but provided limited details. |
| Metric 15: | Number per Group | Unacceptable | × 1 | 4 | The authors mentioned the adaptation of a standard assay method. However, they did not provide details of the number and replicates used per study group. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Low | × 2 | 6 | The reporting was incomplete and it was unclear whether methods were sensitive for the outcome of interest. |
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| Study Citation: | Imperial Chemical Industries Ltd. (1976). Mutagenicity testing with salmonella typhimurium strains on plates, of gases, liquids and solids for Imperial Chemical Industries Limited with attachments | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Genotoxicity-bacterial reverse mutation | | | | | |
| HERO ID: | 4215890 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | The authors did not provide specifics about the execution of the study protocol. | |
| | Metric 18: Sampling Adequacy | Low | × 2 | 6 | Details regarding sampling of outcomes were not fully reported. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable for this study. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Details about the number of organisms used per group were not reported. These deficiencies are likely to have a substantial impact on results. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Although data is available for calculations, the high toxicity seen at two higher doses might impact the analysis. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | High toxicity makes the interpretation of data difficult. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | Although the endpoints of cytotoxicity were defined, the methods of measurements were not fully described or reported. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Study authors reported data for all exposure groups. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.4 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 60: Animal toxicity evaluation results of Doolittle et al., 1987 study on DNA replication and repair

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|--|
| Study Citation: D. J. Doolittle, G. Muller, H. E. Scribner (1987). Relationship between hepatotoxicity and induction of replicative DNA synthesis following single or multiple doses of carbon tetrachloride Journal of Toxicology and Environmental Health, 22(1,1), 63-78 | | | | | |
| Data Type: DNA replication and repair | | | | | |
| HERO ID: 194155 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substance was manufactured by Aldrich Chemical Co. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance was identified as >99% pure. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Appropriate negative controls were administered. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcomes assessed. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Test substance was dissolved just prior to dosing. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Test substance was administered consistently and appropriately. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | 2 groups for DNA repair; 6 groups for replication; liver toxicity was assessed. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method were reported and appropriate. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Health status of mice was not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Some husbandry conditions were not reported (i.e. temperature, humidity, light/dark cycles). |
| Metric 15: | Number per Group | High | × 1 | 1 | 3-6 mice / group were studied. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was reported and sensitive for the outcome of interest. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was consistent across study groups. |
| Metric 18: | Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for outcomes (30-50 cells for UDS; 1000 cells for replication). |
| Metric 19: | Blinding of Assessors | High | × 1 | 1 | Slides were coded and scored without knowledge of treatment. |
| Metric 20: | Negative Control Response | High | × 1 | 1 | Negative control did not elicit response. |
| Domain 6: Confounding / Variable Control | | | | | |

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

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

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

| Study Citation: | R. G. Nath, D. Li, K. Randerath (1990). Acute and long-term effects of carbon tetrachloride on DNA modifications (I-compounds) in male mouse liver Chemico-Biological Interactions, 76(3,3), 343-357 | | | | |
|-------------------------------------|--|---------------------|------|-------|--|
| Data Type: | DNA synthesis | | | | |
| HERO ID: | 6146 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified by chemical name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Manufacturer was reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of test substance were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent vehicle controls were used (same injection volume). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation in corn oil was described. Storage was not reported; however, only a single injection was used. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was administered consistently. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Information was provided to allow calculation of dose (% v/v, ml/kg bw). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single dose was adequate for the outcome of interest. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single dose group; level was not justified. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Route and method were suited to the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and age were reported. The test animal was obtained from a commercial source. Body weight and health status were not reported. Mice were described as retired breeders (10-12 months old). |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | 3-4/group was adequate for the outcome of interest. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment method was reported and sensitive for the outcome of interest. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome was assessed consistently across groups. |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response appears adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | The lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were described and appropriate. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were fully reported across timepoints. |
| Overall Quality Determination [‡] | | High | | 1.5 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 62: Animal toxicity evaluation results of Wacker et al., 2001 study on HNE DNA adduct quantitation

| Study Citation: | M. Wacker, P. Wanek, E. Eder (2001). Detection of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats <i>Chemico-Biological Interactions</i> , 137(3,3), 269-283 | | | | |
|-------------------------------------|---|---------------------|------|-------|--|
| Data Type: | HNE DNA adduct quantitation after ip CCl4 | | | | |
| HERO ID: | 194416 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | Test material was reported by name. |
| | Metric 2: Test Substance Source | High | × 1 | 1 | Test substance source was reported. Lot/batch was not reported, but the composition is not expected to vary. |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | Purity was not reported. |
| Domain 2: Test Design | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | Concurrent untreated control was reported. No vehicle was indicated for CCl4. |
| | Metric 5: Positive Controls | Not Rated | NA | NA | Positive control was not applicable to the study type. |
| | Metric 6: Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Low | × 1 | 3 | It is unclear whether test substance was diluted in a vehicle prior to injection. Injection volume was reported to be small (50 uL). |
| | Metric 8: Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was inferred from text to be consistent across study . |
| | Metric 9: Reporting of Doses/Concentrations | High | × 2 | 2 | Dose were clearly reported. |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were reported and were adequate for the study. |
| | Metric 11: Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Single dose group was adequate for the outcome; dose level was not justified. |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | Exposure route was appropriate for the test . |
| Domain 4: Test Organism | | | | | |
| | Metric 13: Test Animal Characteristics | High | × 2 | 2 | Test animal source and characteristics were reported and appropriate. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Animals husbandry was reported and adequate. |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Number of animals per group was inferred from the text and appeared adequate for statistical . |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment was appropriate for the outcome of interest. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was inferred to be carried out . |
| Continued on next page ... | | | | | |

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| Study Citation: | M. Wacker, P. Wanek, E. Eder (2001). Detection of 1, N2-propanodeoxyguanosine adducts of trans-4-hydroxy-2-nonenal after gavage of trans-4-hydroxy-2-nonenal or induction of lipid peroxidation with carbon tetrachloride in F344 rats <i>Chemico-Biological Interactions</i> , 137(3,3), 269-283 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | HNE DNA adduct quantitation after ip CCl4 | | | | | |
| HERO ID: | 194416 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric was not applicable for the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study type. | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | Negative controls appeared to respond appropriately. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported across groups. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were reported and were appropriate for the data set. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all groups and outcomes. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 63: Animal toxicity evaluation results of Chung et al., 2000 study on HNE DNA adducts in rat liver

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

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| Study Citation: | F. L. Chung, R. G. Nath, J. Ocando, A. Nishikawa, L. Zhang (2000). Deoxyguanosine adducts of t-4-hydroxy-2-nonal are endogenous DNA lesions in rodents and humans: detection and potential sources Cancer Research, 60(6,6), 1507-1511 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | HNE DNA adducts in rat liver | | | | | |
| HERO ID: | 194418 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was described in adequate detail. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | The outcome assessment appeared to be consistent across study groups, except that control animals were sacrificed with the 24 hour time group (no control for the 72 hour time group). | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | Negative control responses were appropriate but had limitations: adducts are also generated endogenously. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No information pertaining to confounding variables in test design were reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | No confounding variables unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical methods were not reported, however data and statistical results were provided and appeared appropriate. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all groups and outcomes. | |
| Overall Quality Determination [‡] | | Medium | | 1.9 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 64: Animal toxicity evaluation results of Wang et al., 1995 study on MDA DNA adducts in hamster liver and kidney

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: M. Y. Wang, J. G. Liehr (1995). Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis Archives of Biochemistry and Biophysics, 316(1,1), 38-46 | | | | | |
| Data Type: MDA DNA adducts in hamster liver and kidney | | | | | |
| HERO ID: 194420 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was reported by name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substance source was reported as Sigma Co. Lot/batch was not reported, but composition is not expected to vary. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent vehicle controls (corn oil) served as negative control. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Not applicable for the study type. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was inferred to be prepared by dilution in corn oil, storage was not reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported in ml/kg and can be converted. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single exposure followed by 4 hours before sacrifice was reported and appropriate for the outcome. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Exposure groups and spacing were not justified by the authors but appeared appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method were appropriate for the test . |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Species, strain, age and commercial source were identified. BOdy weight and health status were not given. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not sufficiently reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | 4/group was sufficient for the outcome of . |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology addressed and was sensitive for the outcome of interest. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was inferred from text to be carried out consistently. |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | Not applicable for the study type. |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study type. |

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| Study Citation: | M. Y. Wang, J. G. Liehr (1995). Lipid hydroperoxide-induced endogenous DNA adducts in hamsters: possible mechanism of lipid hydroperoxide-mediated carcinogenesis Archives of Biochemistry and Biophysics, 316(1,1), 38-46 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | MDA DNA adducts in hamster liver and kidney | | | | | |
| HERO ID: | 194420 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | Negative control responses were reported and appeared appropriate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported across groups. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistics were reported and appropriate for the dataset. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data shown for all . | |
| Overall Quality Determination [‡] | | High | | 1.6 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 65: Animal toxicity evaluation results of Chaudhary et al., 1994 study on M1G -deoxyribose and lipid peroxidation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: A. K. Chaudhary, M. Nokubo, G. R. Reddy, S. N. Yeola, J. D. Morrow, L. J. Blair IA: Marnett (1994). Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver Science, 265(5178,5178), 1580-1582 | | | | | |
| Data Type: M1G -deoxyribose, lipid peroxidation | | | | | |
| HERO ID: 194422 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test material identified by established nomenclature as CCL4; no CASRN was provided. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test material was not reported |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | A concurrent vehicle (corn oil) control was used |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control is not generally used for the study type |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Limited information on preparation (CCL4 was administered in corn oil) and no information on storage were provided in this study. Storage conditions are unlikely to affect the results given the short duration (single exposure) of the study |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Consistency of gavage volumes (0.9mL/kg) across groups was inferred from the text, and the volume was appropriate. Time of day was not reported but unlikely to substantially impact the results. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | The single dose (0.1 mg/kg bw) was clearly reported |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The frequency/duration (single dose) was clearly reported. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Justification for the dose was not reported however, a response was observed. The single dose was acceptable for the outcome of interest |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The exposure route (gavage) was appropriate for the test substance, the study type, and the outcome of interest. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | Test animal species and strain (Sprague-Dawley rats) were reported, but source, sex, age, and starting body weights were not provided. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | The number of animals/group (n = 5) was reported and appropriate for the outcome of interest. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | Methodological details on how MIG residues were assessed were omitted. |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | The consistency of outcome assessment between the treatment and control group cannot be determined due to lack of methodological details. |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Details regarding sampling (extractions from liver) were not reported, but are unlikely to have a substantial impact on results. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not applicable; the outcomes were not subjective. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological response (MIG residue concentration) of the control animals was reported quantitatively and appeared appropriate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, health status, and food/water intake were not reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on health outcomes unrelated to exposure were not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The statistical method used to compare MIG residue levels was reported and appropriate (Wilcoxon rank sum test). |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | MIG residue means were provided with an unspecified measure of error (SD or SEM). |
| Overall Quality Determination [‡] | | Medium | | 2.0 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 66: Animal toxicity evaluation results of Schwarz et al., 1979 study on alkaline elution assay in NMRI mice

| Study Citation: | M. Schwarz, J. Hummel, K. E. Appel, R. Rickart, W. Kunz (1979). DNA damage induced in vivo evaluated with a non-radioactive alkaline elution technique Cancer Letters, 6(4-5,4-5), 221-226 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | alkaline elution assay in NMRI mice | | | | | |
| HERO ID: | 194425 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as CCl4. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Test substance source was not reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent vehicle controls were used. | |
| Metric 5: | Positive Controls | High | × 1 | 1 | Several compounds typically used as positive controls were studied (e.g., DMN, MMS, AAF). | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was inferred to be diluted in vehicle. Storage was not . | |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Exposure administration is inferred to be consistent across study groups | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported in table 1 clearly in ml/kg and can be converted. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were reported and . | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Number of exposure groups and spacing were not justified by the authors but appeared appropriate for the study. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method were appropriate for the test substance. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source of the test animals was not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not . | |
| Metric 15: | Number per Group | High | × 1 | 1 | Number of animals was reported in table 1 (n=5) and is sufficient for . | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome assessment methodology was partially reported, is commonly used, and was sensitive for the outcome of . | |
| Metric 17: | Consistency of Outcome Assessment | Medium | × 1 | 2 | It was inferred that the outcome assessment was carried out . | |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | Not applicable for the study type | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study type |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | Negative control response was reported, however it is unclear if all vehicle controls (and all time points) were combined in table 1. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported across groups. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was not described, however data reported was sufficient for independent analysis. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all groups and . |
| Overall Quality Determination [‡] | | Medium | | 1.9 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 67: Animal toxicity evaluation results of Stewart et al., 1981 study on rat liver DNA damage

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: B. W. Stewart (1981). Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA in vivo Cancer Research, 41(8,8), 3228-3243 | | | | | |
| Data Type: Rat liver DNA damage | | | | | |
| HERO ID: 194464 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified by name. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Test substance source was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent vehicle controls were described in table legend. |
| Metric 5: | Positive Controls | High | × 1 | 1 | Several compounds that could be considered positive controls were assessed (e.g., DEN, BaP, MMS). |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Animal allocation was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was diluted in corn oil, as inferred from table 1 legend. Storage was not reported. |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Exposure administration was assumed to be consistent. |
| Metric 9: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | Doses were reported in a range 200-800 mg/kg in table 1, not specified further. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency (single dose) and duration (4 or 24 hour) were reported |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Unacceptable | × 1 | 4 | Number of groups was not reported. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method are appropriate for the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | Test animal characteristics (sex and strain) were partially reported. Source was not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not sufficiently reported. |
| Metric 15: | Number per Group | Low | × 1 | 3 | Number of animals was reported as pairs. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Low | × 2 | 6 | Outcome assessment methodology appears adequate, but is not commonly used (caffeine elution). |
| Metric 17: | Consistency of Outcome Assessment | Medium | × 1 | 2 | Outcome assessment was inferred through text to be carried out consistently across test groups. |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | Not applicable for the study type. |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | Not applicable for the study type. |
| Continued on next page ... | | | | | |

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| Study Citation: | B. W. Stewart (1981). Generation and persistence of carcinogen-induced repair intermediates in rat liver DNA in vivo Cancer Research, 41(8,8), 3228-3243 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Rat liver DNA damage | | | | | |
| HERO ID: | 194464 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 20: Negative Control Response | Unacceptable | × 1 | 4 | Negative control responses were not reported. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported across groups. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Statistics were not reported and data was not sufficient for an independent statistical analysis | |
| | Metric 24: Reporting of Data | Unacceptable | × 2 | 8 | Data was reported qualitatively in table 2 for each timepoint, but not by dose. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.7 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 68: Animal toxicity evaluation results of Suzuki et al., 1997 study on mouse micronucleus assay

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|---|
| Study Citation: H. Suzuki, N. Hirano, C. Watanabe, Y. Tarumoto (1997). Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood Mutation Research, 394(1-3,1-3), 77-80 | | | | | |
| Data Type: Mouse micronucleus assay for CCl4 | | | | | |
| HERO ID: 194473 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as CCl4 and by CASRN. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported (Wako Pure chemical). Although a lot/batch number was not reported, the test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative (vehicle-only) control groups were reported (assays in bone marrow and peripheral blood). For the peripheral blood micronucleus assay, negative controls were included for each time point. |
| Metric 5: | Positive Controls | Medium | × 1 | 2 | Positive control groups were used (mitomycin C) and positive responses were observed. Positive controls were used at each time point (peripheral blood assay); however, a positive control was not used for the double-dosing experiment (bone marrow assay). |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Animal allocation was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was described (i.e., dissolved in olive oil). Although storage was not reported, this omission is unlikely to impact the results (short-term study). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was inferred to be consistent across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported clearly in Tables 1 (bone marrow test) and 2 (peripheral blood test). |
| Metric 10: | Exposure Frequency and Duration | Medium | × 1 | 2 | The exposure frequency/duration were reported and were appropriate for the outcome of interest (single- or double-dosing for the gavage bone marrow test and single i.p. injection for the peripheral blood test). The time between dosing for the gavage experiment (double-dosing protocol) was not explicitly specified. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Doses were justified with a preliminary acute study (gavage bone marrow test) or based on a previous acute lethality test (i.p. peripheral blood test); the number of groups was consistent with studies of this type. |
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| Study Citation: | H. Suzuki, N. Hirano, C. Watanabe, Y. Tarumoto (1997). Carbon tetrachloride does not induce micronucleus in either mouse bone marrow or peripheral blood Mutation Research, 394(1-3,1-3), 77-80 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Mouse micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194473 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | The exposure route for the bone marrow test was appropriate for the study type (gavage); the route for the peripheral blood test (i.p. injection) is not recommended by study type (not relevant to human exposure); no justification was reported. | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal characteristics were briefly described; animals were obtained from a commercial source. Initial body weights were not reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Most husbandry conditions (temperature, humidity) were reported and were adequate and similar for all groups. Light/dark cycle was not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per group was reported (n =5 per group and/or time point) and appropriate for the outcome of interest. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | In the bone marrow test, the outcome was assessed at only one time point after dosing a single time (two time points are recommended). In the peripheral blood test (single i.p. dose), at least two time points are recommended and were used (48 and 72 hours); the test also evaluated the outcome for peripheral cells at 24 hours (not recommended by study type). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was inferred to be carried out consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Sampling was (1000 erythrocytes per animal) and was adequate to evaluate the outcome of interest for the bone marrow assay and slightly less than recommended for the peripheral blood. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding of assessors was not reported and was not needed as outcomes assessed via Giemsa analysis were not subjective. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control groups were adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No information on confounding variables in test design or procedure were reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | No differences in health outcomes unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analyses were performed; methods were cited to another publication. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all groups and outcomes. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------|---------------------|------|-------|------------------------|
| Overall Quality Determination [‡] | | Medium | | 1.7 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment <i>Toxicologic Pathology</i> , 26(2,2), 247-252 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | DNA adducts for CCl4 | | | | | |
| HERO ID: | 194478 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was clearly identified by name (carbon tetrachloride). | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was identified. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of the test substance was >99.8%; therefore, observed effects are likely due to the test substance itself. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using a negative control group (clearly marked as a vehicle-only control). | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls are not generally used for the study/outcome type. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was described (i.e., dissolved in olive oil), but storage was not reported. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Deficiencies in reporting of administered doses occurred (i.e., 5 mL/kg and no information on animal body weight). | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Animals were administered a single gavage dose of the test substance (appropriate for the outcome of interest). | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Only one dose was used to evaluate DNA adduct formation; however, this dose was sufficient to elicit a positive response at two time points. The study noted that there was an extensive database for hepatotoxicity related to CCl4, and that histopathological liver effects were seen after a single 1 mL/kg dose (but the dose used in this study was 5 mL/kg). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance (oral gavage). | |
| Domain 4: Test Organism | | | | | | |
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| Study Citation: | S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment Toxicologic Pathology, 26(2,2), 247-252 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | DNA adducts for CCl4 | | | | | |
| HERO ID: | 194478 | | | | | |
| Domain | Metric | Rating [‡] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Minor uncertainties in the reporting of test animal characteristics (health status and starting body weight) are unlikely to have a substantial impact on results. The test animals were obtained from a commercial source was appropriate for the outcome of interest. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The reported number of animals per study group was lower than the typical number used (3 males/time point), but sufficient for statistical analysis. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodology was briefly described, and partially cited to another publication. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was evaluated consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 20: Negative Control Response | | × 1 | NA | The biological responses of the negative control groups were adequate (low incidence of adducts). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on confounding variables were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analysis was not described (e.g., test used); but statistically analyzed data were reported. From the data presented in Figure 2 (and n = 3), means and measures of variance could be estimated. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data is presented in Figure 2 (control and treatment groups at each time point). | |
| Overall Quality Determination [‡] | | Medium | | 0.0 | | |
| Extracted | | Yes | | | | |
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Study Citation: S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai, T. Shirai (1998). Immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment Toxicologic Pathology, 26(2,2), 247-252

Data Type: DNA adducts for CCl4

HERO ID: 194478

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: F. Tombolan, D. Renault, D. Brault, M. Guffroy, F. Perin, V. Thybaud (1999). Effect of mitogenic or regenerative cell proliferation on lacz mutant frequency in the liver of MutaTMMice treated with 5, 9-dimethyldibenzo[c,g]carbazole Carcinogenesis, 20(7,7), 1357-1362 | | | | | |
| Data Type: Mitogenic and regenerative cell proliferation | | | | | |
| HERO ID: 194500 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the substance was identified as Prolabo (Paris, France). |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Concurrent negative control group was included (vehicle only), however mice in control group were sacrificed on day 1 and 7 and pooled. Experimental group were sacrificed on day 1, 2, 3, 4 and 7. This is unlikely to have a substantial impact on results. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive responses were verified in small intestine tissue within the same animal and/or were elicited with other substances run. Results from positive control (small intestine) samples were not reported. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of test animals was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation of test substance was reported. Storage of test substance was not reported, but this is appropriate given the study design (single-dose administration). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Dose concentration was reported to be 80 mg/kg. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were reported and appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Only one dose (80mg/kg) was used in this study therefore dose-responses were not reported. However, positive results were obtained from this dose, so it was considered to be an adequate dose for the outcome of interest. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route was appropriate (oral gavage). |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strains, sex, age, and commercial source were reported. Health status and beginning body weights were not provided. Details regarding the unique genetic feature (bacterial LacZ reporter gene) of the Muta(TM)Mice were described. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Husbandry conditions were reported. |

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| Study Citation: | F. Tombolan, D. Renault, D. Brault, M. Guffroy, F. Perin, V. Thybaud (1999). Effect of mitogenic or regenerative cell proliferation on lacZ mutant frequency in the liver of MutaTMMice treated with 5, 9-dimethyldibenzo[c,g]carbazole Carcinogenesis, 20(7,7), 1357-1362 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Mitogenic and regenerative cell proliferation | | | | | |
| HERO ID: | 194500 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | For CD2F1 mice studies, n = 4 mice/treatment group. For Muta(TM)Mice studies, n = 5 mice/treatment group. For negative controls, n = 2-3 mice. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome methodology was partially reported and cited elsewhere. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was reported and consistent across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | BrdU-immunopositive nuclei: 3000-35000 hepatocellular nuclei scored. Mutant frequency determination: at least 200,000 plaque-forming units by animal were recorded. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not necessary for these studies. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control response was appropriate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial body weights and food/water intake were not reported however it is not likely to have a significant impact on results. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Health outcomes unrelated to test substance were not reported although it unlikely to have a substantial impact on results. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was performed by Student's t-test and appropriate. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all outcomes in Figures/ Tables and text. | |
| Overall Quality Determination [‡] | | High | | 1.4 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | J. C. Mirsalis, B. E. Butterworth (1980). Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens <i>Carcinogenesis</i> , 1(7,7), 621-625 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | In vivo UDS | | | | | |
| HERO ID: | 194512 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source was identified as Fisher Scientific Co. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity or grade of test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative control was included (vehicle). | |
| Metric 5: | Positive Controls | High | × 1 | 1 | Dimethylnitrosamine and acetylaminofluorene were both utilized as positive control substances to test the novel method of assessing UDS ex vivo. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Test substance was prepared in corn oil. Storage was not reported, but this is appropriate given the study design (single-dose administration). | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across treatment groups (negative controls and two doses of CCl4). | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses are reported as 10 and 100 mg/kg. | |
| Metric 10: | Exposure Frequency and Duration | Low | × 1 | 3 | Exposure was reported as 2 hr. This sampling time after test substance administration appeared to be appropriate due to the positive response from DMN and AAF at this timepoint. However, current standards recommend a longer timepoint, such as 12-16 hours; it is unclear why DMN- and AAF-treated animals were sampled at 12, 24, and 48 hours, while CCl4-treated animals were not. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Unacceptable | × 1 | 4 | Only 2 doses used. Not clear if highest dose was high enough to elicit response. There was no justification for the selection of the doses. The highest dose tested should produce signs of toxicity such that higher doses would be expected to result in mortality. There was no indication that the test animals administered the highest dose of CCl4 (100 mg/kg) exhibited signs of toxicity. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Route of exposure was appropriate. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Age and health status are not reported, it is unlikely to have a substantial impact on results | |
| Continued on next page ... | | | | | | |

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| Study Citation: | J. C. Mirsalis, B. E. Butterworth (1980). Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an in vivo-in vitro assay for potential carcinogens and mutagens <i>Carcinogenesis</i> , 1(7,7), 621-625 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | In vivo UDS | | | | | |
| HERO ID: | 194512 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions are not sufficiently reported. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | It appears that each "experiment" referred to in Table II refers to a single animal. Thus, for 10 mg/kg, n = 1. For 100 mg/kg, n = 2. The number per group is considered to be lacking. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Methodology is partially reported and cited elsewhere (Bermudez, 1979). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was carried out consistently across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | 50 cells were scored for each of 3 slides per group, which is in line with current guidelines. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Automated measurements were used. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response was adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no confounding variables in test design reported. Initial body weights were 200-250 g | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Statistical analysis was not conducted. Data are presented as mean ± SE and SD. However, independent statistical analysis could not be completed appropriately from the mean, SD, and n because the study conflated slide-to-slide variability with experiment-to-experiment variability. The data appeared to be inappropriately pooled (using n = 300 rather than n = 2) and thus resulted in reporting smaller standard deviations than reality. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported in Table II. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.7 | | |
| Extracted | | No | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 72: Animal toxicity evaluation results of Mirsalis et al., 1989 study on DNA replication and UDS in hepatocytes

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: J. C. Mirsalis, C. K. Tyson, B. E. Butterworth (1982). Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay Environmental and Molecular Mutagenesis, 4(5,5), 553-562 | | | | | |
| Data Type: DNA replication and UDS in hepatocytes | | | | | |
| HERO ID: 10063 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride (CCl4). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of test substance was identified as Fisher Scientific Co. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance was identified as ACS grade. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Appropriate concurrent negative controls were included. |
| Metric 5: | Positive Controls | High | × 1 | 1 | Positive responses were elicited by 2-AAF and MMS run concurrently. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was prepared in corn oil. Time frame from when solution was made until administered is not given, unclear if storage was necessary. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported in Table I and II (40 and 400 mg/kg). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequencies and duration were reported and appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Ability of CCl4 to induce DNA replication was only performed at one dose (400mg/kg). DNA repair (UDS) was evaluated at two doses (40 and 400 mg/kg) |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and appropriate for the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Species, strain, sex, initial body weight, health status and commercial source were reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | Medium | × 1 | 2 | Number of animals/groups was reported to be 2-4 in Figure 1 and Table I and II. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Methodology for UDS was described, however assessment methodology for DNA replication was not described. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was consistent across groups.. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Random selection of 50 morphological unaltered cells were counted. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Study used an ARTEK Model 880 colony counter and data were fed directly into a Digital Equipment Corp Vax11/780 computer. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Responses of the negative control group were adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weights and food/water intake were not reported for each study group. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Data is presented at mean± SE. |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | It is unclear if data points were not reported due to lack of details in methods. Figure 1 shows time point for 24hr and 48 hr that are not reported in Table II. The dose used to generate Figure 1 was also not reported. It is not clear if other doses/ timepoints were used to investigate DNA replication (Table 1). |
| Overall Quality Determination [‡] | | High | | 1.6 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 73: Animal toxicity evaluation results of Morita et al., 1997 for micronucleus assay

| Study Citation: | T. Morita, N. Asano, T. Awogi, Y. F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS Mutation Research, 389(1,1), 3-122 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194532 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name (carbon tetrachloride). A CASRN (56-23-5) was also provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was reported. A lot number (Lot # SAK7972) was provided. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of the test substance was reported (99.8%); therefore, effects are likely due to the test substance itself. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using a concurrent negative (vehicle-only) control group for the bone marrow assay, and a sample before treatment (0 hours) as a negative control in the peripheral blood assay. | |
| Metric 5: | Positive Controls | Medium | × 1 | 2 | The study authors reported using a positive control (mitomycin C) in the micronucleus assays. It was indicated that positive responses were observed, but data were not shown. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance was dissolved in olive oil. Lack of reporting with respect to storage conditions is not likely to have a substantial impact on results. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity in Table 5. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration were reported (i.e., the number/spacing of treatments) and were appropriate for the study type. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups (3 doses [plus negative control for the bone marrow assay]) was reported. Doses were selected based on the outcome of preliminary dose-finding tests. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The bone marrow assay was performed by oral gavage; the peripheral blood assay was performed by i.p. injection (not considered a relevant route of human exposure). | |
| Domain 4: Test Organism | | | | | | |
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| Study Citation: | T. Morita, N. Asano, T. Awogi, Y. F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS Mutation Research, 389(1,1), 3-122 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194532 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Low | × 2 | 6 | The source of the test animals was not reported. The strain, sex, and age of the mice used in the study were reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per study group (5 males) was reported, appropriate for the study type and outcome analysis. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Details regarding outcome assessment methodology were limited. In general, the timing of assessments were sensitive for the outcome of interest (two sampling times in the recommended time frame for the peripheral blood assay; one sampling time in the recommended time frame for the bone marrow assay after two gavage doses). The single gavage dose bone marrow assay only one sampling time (two are recommended). | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Although limited details are reported, the data tables indicate that outcomes were assessed consistently across study groups (at the same times after initial exposure). | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Sampling was reported (1000 polychromatic erythrocytes or reticulocytes/animal). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type (blinding not reported). | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | The biological responses of the negative control group were adequate (low incidences of micronucleus formation). The study authors noted that historical control data were not always available, and that control data were judged subjectively. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Confounding variables in test design and procedures were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Data are presented as mean ±SD in Table 5. Statistical analyses were performed. Micronucleus frequency in treated groups was compared to concurrent controls using a conditional binomial test; the does-response was evaluated using a Cochran-Armitage trend test. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for outcomes is presented in Table 5 (by assay, time point, exposure group). | |

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--|--------|---------------------|------------------|-------|------------------------|
| Overall Quality Determination [‡] | | Medium | | 1.7 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 74: Animal toxicity evaluation results of Uryvaeva et al., 1995 study on a micronucleus assay

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| Study Citation: I. V. Uryvaeva, G. V. Delone (1995). An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study Mutation Research, 334(1,1), 71-80 | | | | | |
| Data Type: Micronucleus assay for CCl4 | | | | | |
| HERO ID: 194598 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name (carbon tetrachloride). |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | CCl4-treated mice were compared to mice with partial hepatectomy. Negative controls were not employed. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study type. The aim of the study was to compare micronuclei formation (and its age dependence) following mitotic stimulation via CCl4 or partial hepatectomy. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Unacceptable | × 1 | 4 | There was no mention of the method and equipment used to generate the test substance. |
| Metric 8: | Consistency of Exposure Administration | Unacceptable | × 1 | 4 | Critical exposure details (e.g., methods for generating atmosphere in inhalation studies) were not reported. In addition, animals were evaluated at 2.5 months after partial hepatectomy [PH] or CCl4 treatment only; at the other time points, there was only one condition or the other (PH at 5 months and CCl4 treatment at 7 months). |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Actual concentrations and/or analytical methods were not reported. The study indicated the range of exposure (0.05 to 0.1 mL). |
| Metric 10: | Exposure Frequency and Duration | Low | × 1 | 3 | The duration of exposure was reported (15 minutes). The duration of the study was likely not sufficient to induce micronuclei induction. The study was intended to evaluate the ability of CCl4 to induce mitosis in the liver (and was based on the assumption that the test substance is non-genotoxic). |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | A single dose level was used to characterize the age-dependence of micronuclei induction in hepatocytes. The dose was adequate for that purpose, but the concentration was not likely high enough for the purpose of evaluating the genotoxicity of CCl4. |
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| Study Citation: | I. V. Uryvaeva, G. V. Delone (1995). An improved method of mouse liver micronucleus analysis: an application to age-related genetic alteration and polyploidy study Mutation Research, 334(1,1), 71-80 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194598 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Low | × 1 | 3 | Animals were exposed to the test substance in a 5L sealed box; distribution of the the substance in the whole-body chamber was not reported (not clear if dynamic). | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Minor uncertainties in the reporting of test animal characteristics (health status, starting body weight) are unlikely to have a substantial impact on results. The test animals were obtained from a nursery farm, and the test species was an appropriate animal model. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | It appears that there were at least 5 males/group, which is adequate for the study type. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Methodology related to micronucleus examination was described and partially cited to another publication. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes appeared to be assessed consistently after exposure. | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | The study indicated that 1000 (animals aged 5 and 7 months) to 2000 cells/animal (animals aged 2.5 months) were screened for micronuclei. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the PH group were adequate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions of study groups were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Data were presented as means +/- SD. Data were presented in a form amenable to independent statistical analysis. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported by exposure group. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.4 | | |
| Extracted | | No | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 75: Animal toxicity evaluation results of Van Goethem et al., 1995 study on a micronucleus assay

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

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| Study Citation: | F. Van Goethem, J. de Stoppelaar, B. Hoebee, M. Kirsch-Volders (1995). Identification of clastogenic and/or aneugenic events during the preneoplastic stages of experimental rat hepatocarcinogenicity by fluorescence in situ hybridization <i>Carcinogenesis</i> , 16(8,8), 1825-1834 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194600 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The strain, age, and weights (range) of the test species were reported. Minor uncertainties in the reporting of test animal characteristics (e.g., health status) are unlikely to have a substantial impact on results. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Some husbandry conditions were reported (standard conditions with 12 hour light/dark schedule). Minor uncertainties (temperature and humidity) are not expected to impact the study results. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The reported number of animals per study group was lower than the typical number used in studies of the same or similar type (3 males/group). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment were partially described and cited elsewhere. The outcome was assessed only 72 hours after treatment (at least two time points are recommended after a single exposure). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling was adequate (about 4000 hepatocytes/animal). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric was not applicable to the study type (blinding not reported). | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological response of the negative control group was adequate (low incidence of micronuclei; mean = 1.5%). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions (e.g., food/water intake) were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was performed for data related to CCl4 treatment alone (p-values were provided in the text). The data (Table 1) were presented as means +/- standard deviation for n =3 animals, which is also amenable to independent analyses. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported by exposure group. | |
| Overall Quality Determination [‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| Study Citation: F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis <i>Carcinogenesis</i> , 14(11,11), 2397-2406 | | | | | |
| Data Type: Micronucleus assay for CCl4 | | | | | |
| HERO ID: 194601 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name (carbon tetrachloride). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was reported. Although a batch/lot number was not specified, the test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity/grade of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | The study reported using concurrent negative control groups. There were CCl4 and CCl4+NaCl treatment groups, which were compared to control (presumably untreated but not clearly specified) and NaCl-treated control groups. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive control was used, but treatment-related positive responses were observed (the test is capable of detecting a positive response). |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported; the study only indicated that animals were divided into 10 groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of test substance was described, however details on storage conditions are not. It is unlikely to have a substantial impact on results. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | The dose was reported as 2mL/kg. A dose in mg/kg was not provided, and only a range of initial body weights (i.e., 200 to 220 g) was reported. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration (i.e., single gavage dose) were reported and were appropriate for the study type. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups was reported (1 plus controls). The dose was presumably selected based on previous studies. The number of dose groups is fewer than that recommended by study type; however, the aim of this study was to evaluate micronuclei formation during carcinogenesis induced by initiation, promotion, and CCl4 treatment. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route of exposure was reported and appropriate for the study type. |
| Domain 4: Test Organism | | | | | |

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| Study Citation: | F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis <i>Carcinogenesis</i> , 14(11,11), 2397-2406 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194601 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The strain, age, and weights (range) of the test species were reported. Minor uncertainties in the reporting of test animal characteristics (e.g., health status) are unlikely to have a substantial impact on results. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Some husbandry conditions were reported (standard conditions with 12 hour light/dark schedule). Minor uncertainties (temperature and humidity) are not expected to impact the study results. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The reported number of animals per study group was lower than the typical number used in studies of the same or similar type (2 males/group in CCl4, CCl4+NaCl, control, and NaCl only groups). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome methodology was partially described and partially cited to other publications (e.g., hepatocyte isolation). Although more than one time point for analyses after a single treatment is recommended, initial experiments were conducted to determine the optimal time point for scoring micronuclei. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling was reported and adequate (at least 4000 hepatocytes scored per animal). | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | This metric was not applicable to the study type (blinding not reported). | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | The biological response of the negative control group was adequate (low incidence of micronuclei; mean = 0.2 to 1.0%). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions (e.g., food/water intake) were not reported. Inter-individual differences likely contributed to differences in response. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | Statistical methods used in the study were described; however, it does not appear that statistics were applied to data for CCl4 treatment alone (or +NaCl; there were only 2 animals per group. | |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Data were reported by exposure group (individual animal). However, data were shown as % incidence (raw numbers of micronuclei not reported). | |
| Overall Quality Determination [‡] | | Medium | | 1.9 | | |
| Extracted | | Yes | | | | |
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Study Citation: F. Van Goethem, M. A. Ghahroudi, P. Castelain, M. Kirsch-Volders (1993). Frequency and DNA content of micronuclei in rat parenchymal liver cells during experimental hepatocarcinogenesis *Carcinogenesis*, 14(11,11), 2397-2406
 Data Type: Micronucleus assay for CCl4
 HERO ID: 194601

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | G. Varela-Moreiras, E. Alonso-Apperte, M. Rubio, M. Gasso, R. Deulofeu, L. Alvarez, J. Caballeria, J. Rodes, J. M. Mato (1995). Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment Hepatology, 22(4 Pt 1,4 Pt 1), 1310-1315 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | DNA methylation for CCl4 | | | | | |
| HERO ID: | 194604 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name and formula. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of test substance were not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Control rats were untreated (not clear whether vehicle was used). | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Unacceptable | × 1 | 4 | No details were provided on preparation of test substance for injection or storage. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure appeared consistent. | |
| Metric 9: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | Dose was unknown. Injection volume was reported as mL/kg, but it is not clear whether CCl4 was dissolved in vehicle prior to injection. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Duration and frequency was appropriate for the study type (2x/week for 3 weeks). | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single exposure group; dose not provided or justified. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Intraperitoneal injection was appropriate for the test substance, but not relevant to human exposure. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source of the test animal was not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Temperature and light/dark cycle were reported and adequate. Humidity was not reported. | |
| Metric 15: | Number per Group | High | × 1 | 1 | The number of animals (5/group) is appropriate for the outcome of interest. | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment reported and was sensitive for the outcome of interest. | |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across groups. | |
| Metric 18: | Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| Continued on next page ... | | | | | | |

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| Study Citation: | G. Varela-Moreiras, E. Alonso-Apperte, M. Rubio, M. Gasso, R. Deulofeu, L. Alvarez, J. Caballeria, J. Rodes, J. M. Mato (1995). Carbon tetrachloride-induced hepatic injury is associated with global DNA hypomethylation and homocysteinemia: effect of S-adenosylmethionine treatment <i>Hepatology</i> , 22(4 Pt 1,4 Pt 1), 1310-1315 | | | | |
|--|--|----------------------------|------------------|-------|--|
| Data Type: | DNA methylation for CCl4 | | | | |
| HERO ID: | 194604 | | | | |
| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response appeared appropriate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food/water intake were not reported across groups. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistics were reported and appropriate. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were adequately reported. |
| Overall Quality Determination [‡] | | Unacceptable ^{**} | | 2.2 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 78: Animal toxicity evaluation results of Crebelli et al., 1999 study on a micronucleus assay

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

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| Study Citation: | R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J. A. Vericat (1999). Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test Mutagenesis, 14(2,2), 207-215 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Micronucleus assay for CCl4 | | | | | |
| HERO ID: | 194679 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | CrI:CD-1 (ICR) BR mice (5/sex/dose) were used. Weights were approx. 25-35 g at the time of sacrifice. Animals were purchased from Charles River Italia S.p.A. (Calco, Lecco, Italy). Some details were lacking (health status and age) although are not expected to have a substantial impact on results. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Husbandry conditions were reported - 5 animals per cage at 22 degrees C with a relative humidity of 55% and a dark/light cycle of 12 hours. Animal care followed Directive 86/609/EEC. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals used was reported (5/sex/dose) and was appropriate for the study type. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome methodology was reported and was sensitive for the outcome of interest. Bone marrow was assessed at two time points after a single injection dose (as recommended by the study type). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes appeared to be assessed consistently across dose groups, as reported in Table III. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to this study design. The study indicated that slides were scored by 1 to 2 experienced readers. A single reader read each set of slides to minimize bias. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Vehicle controls responded appropriately (results provided in Table III). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study group. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was conducted and appropriate (χ^2 test and t-test). Additionally, sufficient data were provided to conduct independent analyses. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Results for each dose group and control group by sex were reported (Table III). | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |
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Study Citation: R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J. A. Vericat (1999). Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test *Mutagenesis*, 14(2,2), 207-215
 Data Type: Micronucleus assay for CCl4
 HERO ID: 194679

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: H. J. Curtis, J. Tilley (1968). Chromosome aberrations in liver forced to regenerate by chemical or surgical methods <i>Journals of Gerontology</i> . Series A: Biological Sciences and Medical Sciences, 23(2,2), 140-141 | | | | | |
| Data Type: CAs in mouse liver for CCl4 | | | | | |
| HERO ID: 194696 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of the test substance were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | CCl4-treated mice were compared to mice with partial hepatectomy. Negative controls were not employed. |
| Metric 5: | Positive Controls | Unacceptable | × 1 | 4 | Half of the animals were given X-rays; however, animals were also given CCl4 or underwent partial hepatectomy. No X-ray only group was used. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | it is inferred that a small volume of undiluted CCl4 was injected subcutaneously a single time (no vehicle was described). |
| Metric 8: | Consistency of Exposure Administration | Low | × 1 | 3 | Minimal details were reported regarding exposure (72h prior to sacrifice). |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | Assuming no dilution of test substance, dose can be calculated from mL/g bw. |
| Metric 10: | Exposure Frequency and Duration | Low | × 1 | 3 | Single injection may not be sufficient to show CAs at later time points (10 and 21 weeks of age; injected at 6 weeks) |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single dose; not justified by the authors and not clear if dose was high enough. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Route and method were appropriate for the test substance, but not environmentally relevant. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Species, strain, sex, age and commercial source were reported. Health status and body weight were not given. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | The number of animals used (5/group) is adequate. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Not Rated | NA | NA | The outcome assessment methods were cited to another publication (Stevenson & Curtis, 1961). |

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| Study Citation: | H. J. Curtis, J. Tilley (1968). Chromosome aberrations in liver forced to regenerate by chemical or surgical methods <i>Journals of Gerontology. Series A: Biological Sciences and Medical Sciences</i> , 23(2,2), 140-141 | | | | |
|--|--|---------------------|------|-------|--|
| Data Type: | CAs in mouse liver for CCl4 | | | | |
| HERO ID: | 194696 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | The outcome assessment methods were cited to another publication (Stevenson & Curtis, 1961). |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | 100 figures were scored per animal (500 per group). |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | Cells were scored blind. |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | Negative controls were not used. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported for each group. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Means +/- SEM were provided in the figure. |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Data from the 72 hour sacrifice were not presented. |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.5 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 80: Animal toxicity evaluation results of Sasaki et al., 1998 for in vivo Comet assay

| Study Citation: | Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibasi, K. Yoshida, Q. Y. Su, N. Matsusaka, S. Tsuda (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 419(1-3,1-3), 13-20 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | In vivo Comet assay for CCl4 | | | | | |
| HERO ID: | 38908 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (CCl4). | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Concurrent negative control groups were included (untreated controls). It was stated that previous studies from the laboratory showed no difference between untreated and concurrent vehicle (olive oil) treated controls. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation of the test substance was briefly reported. Storage of the test substance was not reported (single-dose administration). | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was reported to be consistent across treatment groups. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity. | |
| Metric 10: | Exposure Frequency and Duration | Low | × 1 | 3 | The exposure was a single-dose administration, which is lower than the guideline suggests (at least two administrations). It is possible that this resulted in some false negatives across the various organs and timepoints tested. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups and dose spacing for CCl4 were appropriate. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were appropriate for the test substance. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The species, strain, age, sex, and commercial source of the test animals were reported. The starting body weight range of the test animals was not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Husbandry conditions were adequate, appropriate, and consistent. | |
| Continued on next page ... | | | | | | |

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| Study Citation: | Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibasi, K. Yoshida, Q. Y. Su, N. Matsusaka, S. Tsuda (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 419(1-3,1-3), 13-20 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | In vivo Comet assay for CCl4 | | | | | |
| HERO ID: | 38908 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per treatment group was adequate and appropriate for this study design (n = 4). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for this endpoint. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment methodology was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | Sampling was lacking for the outcome of interest (50 nuclei per organ per animal). Test guideline suggests 150 nuclei per animal. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative responses were observed in negative controls. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Starting body weights were not reported. Respiratory rates and food/water consumption were not reported, but this is appropriate given the study design. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | No deaths or health outcomes unrelated to the exposure were reported for this experiment. Histopathological lesions of the liver, including necrosis, were observed at the two highest doses of CCl4 at 24 hours. These lesions were considered to be related to cytotoxicity induced by the test substance, as the changes were dose related. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The data were appropriately analyzed by one-way ANOVA with Dunnett's post-hoc test. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | All data were reported adequately. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 81: Animal toxicity evaluation results of Higami et al., 2004 study on mouse and rat liver DNase and DNA fragmentation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: Y. Higami, T. Tsuchiya, K. To, T. Chiba, H. Yamaza, D. Shiokawa, S. Tanuma, I. Shimokawa (2004). Expression of DNase gamma during Fas-independent apoptotic DNA fragmentation in rodent hepatocytes Cell and Tissue Research, 316(3,3), 403-407 | | | | | |
| Data Type: HERO ID: 194726 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | test substance identified by name |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | test substance source was not reported |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | test substance purity was not reported |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | concurrent negative (vehicle) controls were reported |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Not applicable for the study type |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | allocation of animals was not described |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | test substance was prepared in mineral oil; storage was not reported, but this is unlikely to affect results given that the study was short term in duration (single exposure) |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | exposure were administered consistently across groups |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | dose reported clearly as 0.25 g/100 g BW |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | frequency was a single administration, with sacrifice 8 or 24h post injection |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | number of exposure groups and dose spacing was not justified by study authors but appeared sufficient given that the expected response (necrosis with DNA fragmentation) was observed |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | exposure route was appropriate for the test substance |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | test animal characteristics (pathogen free, age, sex, strain, species) were reported and animals were from a commercial source (Charles River Japan) |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Not Rated | NA | NA | animal husbandry conditions were cited to another publication without further details |
| Metric 15: | Number per Group | High | × 1 | 1 | number of animals per group was reported (6 treated and 5 control) and adequate for study type |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | outcome assessment was cited to another publication and partially described and appeared appropriate for the outcome of interest |
| Continued on next page ... | | | | | |

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

Data Type:

HERO ID: 194726

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Outcome assessment was cited to another publication and briefly described; appeared consistent across groups |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Results obtained by counting 200 microscopic fields (~20,000 hepatocytes each) per animal |
| | Metric 19: Blinding of Assessors | Low | × 1 | 3 | blinding was not reported. One investigator performed all assessments. |
| | Metric 20: Negative Control Response | Unacceptable | × 1 | 4 | negative control response for CCL4 experiment was not reported or noted in text |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial body weight and food/water intake were not reported but unlikely to significantly impact DNA fragmentation results |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | outcomes unrelated to exposure were not reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | statistical methods were reported and appropriate for the data |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Results for CCL4 group described qualitatively in text; results for negative controls not reported. |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.8 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 82: Animal toxicity evaluation results of Barbin et al., 1983 study on DNA damage in rat liver

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|-----------------------|-------|---|
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | outcome assessment methodology was sensitive for the outcome of interest and described in detail. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | outcome assessment was consistent across study groups |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | not applicable for the study type |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | not applicable for the study type |
| | Metric 20: Negative Control Response | High | × 1 | 1 | negative controls response was reported and appeared to be adequate |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No differences in initial body weight or food/water intake were reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | There was no attrition and no health outcomes unrelated to exposure were reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | statistical analysis was reported and appropriate for the data |
| | Metric 24: Reporting of Data | High | × 2 | 2 | data were reported for all outcomes and groups |
| Overall Quality Determination [‡] | | High | → Medium [§] | 1.5 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | the outcome assessment methodology addressed the outcome of interest, and was described in some detail with reference to published methods |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | outcome assessment was described. Table indicates that the amount of DNA analyzed for methylated guanine was much lower in the treated animals (4 mg) compared with controls (10 mg) |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Livers from 2 animals/group pooled for DNA extraction. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | not applicable to the study type |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | negative control responses were reported and appeared adequate (not detected) but limits of detection were not reported |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial body weight was reported; food and water intake were not. However, given the brief duration of the study (single dose with sacrifice 12 hr later the lack of reporting is not likely to significantly impact results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | no health outcomes unrelated to exposures were reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | not applicable for the study type |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | data were from pooled sample (livers from 2 animals/group) and were reported for all outcomes |
| Overall Quality Determination [‡] | | Medium | | 1.8 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 84: Animal toxicity evaluation results of Ikegwuonu et al., 1991 study on DNA damage and repair in rat liver

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | outcome assessment methodology was described in detail with some reference to published methods. Outcome assessment methods were appropriate for the outcome of interest. The duration between treatment and sacrifice was brief (1 hr) relative to recommendations (2-4 or 12-16 hr) but a clear positive response was seen. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | outcome assessment protocol was reported and assessment carried out consistently across all study groups |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | 5 ml hepatocyte suspension containing 1 x 10 ⁶ cells/ml, in triplicate |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | not applicable for the study type |
| | Metric 20: Negative Control Response | High | × 1 | 1 | negative control responses were reported and appropriate |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions (bw, food or water intake) of animals in each study group not reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | data on health outcomes unrelated to exposure were not reported |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods were reported and adequate for the data set |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported for all groups and outcomes including means, SEs, and number determinations |
| Overall Quality Determination [‡] | | High | | 1.6 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 85: Animal toxicity evaluation results of Iwai et al., 2002 study on oxidative DNA damage in rat liver

| Study Citation: | S. Iwai, R. Karim, M. Kitano, T. Sukata, W. Min, K. Morimura, H. Wanibuchi, S. Seki, S. Fukushima (2002). Role of oxidative DNA damage caused by carbon tetrachloride-induced liver injury -- enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats Cancer Letters, 179(1,1), 15-24 | | | | |
|-------------------------------------|--|---------------------|------|-------|---|
| Data Type: | Oxidative DNA damage in rat liver | | | | |
| HERO ID: | 194769 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | test substance was identified by name |
| | Metric 2: Test Substance Source | High | × 1 | 1 | test substance source was reported (Wako Pure Chemical Industries, Osaka, Japan). |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | test substance purity was not reported |
| Domain 2: Test Design | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | concurrent negative vehicle controls were reported |
| | Metric 5: Positive Controls | Not Rated | NA | NA | not applicable for the study type |
| | Metric 6: Randomized Allocation | Low | × 1 | 3 | method of animal allocation was not reported |
| Domain 3: Exposure Characterization | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was prepared in a corn oil vehicle but it is unclear if prepared fresh for each dose. Storage was not reported |
| | Metric 8: Consistency of Exposure Administration | High | × 1 | 1 | exposure administration (e.g., sc injection volume) was consistent across groups |
| | Metric 9: Reporting of Doses/Concentrations | High | × 2 | 2 | dose was reported in ml/kg body weight (0.25) and can be converted to mg/kg/day |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | exposure frequency (2x week) and duration (1 wk) were reported and adequate for the study |
| | Metric 11: Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | One dose group was used. The dose was not justified by authors. A positive response was observed with the one dose, indicating that it was high enough to elicit a response (oxidative DNA damage). |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | exposure route and method of exposure were reported, but location of s.c. administration was not. Route and method were acceptable for the test substance |
| Domain 4: Test Organism | | | | | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Reported test animal characteristics include species, strain, sex, and age; body weight and health condition were not reported. Animals obtained from a commercial source (Charles River Japan) |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Most animal husbandry conditions reported (temperature, RH, and light cycle; housing conditions were not reported), adequate, and similar across groups. |
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| Study Citation: | S. Iwai, R. Karim, M. Kitano, T. Sukata, W. Min, K. Morimura, H. Wanibuchi, S. Seki, S. Fukushima (2002). Role of oxidative DNA damage caused by carbon tetrachloride-induced liver injury -- enhancement of MeIQ-induced glutathione S-transferase placental form-positive foci in rats Cancer Letters, 179(1,1), 15-24 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Oxidative DNA damage in rat liver | | | | | |
| HERO ID: | 194769 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | Number of animals per group was reported (5) and was adequate for the outcome analysis | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome assessment methodology was briefly described and cited to published method. The methodology was appropriate. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was described and consistent across study groups | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | not applicable for the study type | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | not applicable for the study type | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | negative controls responded appropriately | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Initial body weights and food and water intake were not reported but these are unlikely to significantly impact the results | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on health outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | statistical methods were reported and adequate for the data set | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | 8-OHdG levels in rat liver were reported graphically with error bars for all groups | |
| Overall Quality Determination [‡] | | High | | 1.6 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 86: Animal toxicity evaluation results of Bermudez et al., 1982 study on in vivo DNA damage in hepatocytes from treated rats

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| Study Citation: E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679 | | | | | |
| Data Type: in vivo DNA damage in hepatocytes from treated rats | | | | | |
| HERO ID: 194786 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (CCl4). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was identified (Fisher Scientific Co). The product number and batch/lot number were not reported; however the material is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative solvent (corn oil) controls were used |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive control was used; however, treatment-related positive responses were observed for other chemicals tested in the study demonstrating that the test is capable of detecting a positive response. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Animal allocation methodology was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance was reported to have been prepared in corn oil. The storage of the test substance was not reported; however, it was a single administration so storage is unlikely to affect results. |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Details of exposure administration were reported, but with limited details. Gavage volume was not excessive (0.4 mL/100 g bw). Animals were treated with a single exposure, so these limitations are unlikely to have a substantial impact on results. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Administered dose was reported without ambiguity (400 mg/kg). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were appropriate for this endpoint; single oral dose |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Only one dose was tested. The administered dose level was not justified, and results were all negative; it is not certain if a higher dose would elicit a response for this outcome. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The exposure route was appropriate for the test substance (gavage, in corn oil) |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and weight were reported. The commercial source was reported (Charles River Breeding labs). The health status and age were not reported. The test species and strain were an appropriate animal model for the evaluation of this endpoint |

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| Study Citation: | E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | in vivo DNA damage in hepatocytes from treated rats | | | | | |
| HERO ID: | 194786 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported (only feed type reported) to evaluate if husbandry was adequate. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The number of animals per group (2 animals/group) was lower than the typical number used in similar types of studies | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology (alkaline elution) was reported and appropriate for the endpoint of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for the outcomes of interest (cell concentration: 1x10+6/ml) | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for this study type | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control groups were adequate | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food and water intake were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | Statistical analysis between treated and control groups was not conducted. The results indicate if a treatment is considered negative or positive. Statistical analysis may not be necessary for this study. There were negative findings for CCl4-treated groups at all time points. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | DNA damage results were presented quantitatively for all time points for treated animal. Results for solvent controls were pooled across both water controls and corn oil controls (appropriate for CCL4 experiment) and time points. It is unclear whether the quantitative results for alkaline elution, given in % of control, were based on pooled or individual control group results. | |
| Overall Quality Determination [‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |
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Study Citation: E. Bermudez, J. C. Mirsalis, H. C. Eales (1982). Detection of DNA damage in primary cultures of rat hepatocytes following in vivo and in vitro exposure to genotoxic agents Environmental Mutagenesis, 4(6,6), 667-679
 Data Type: in vivo DNA damage in heptocytes from treated rats
 HERO ID: 194786

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plataras, Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | DNA adducts for CCl4 | | | | | |
| HERO ID: | 194788 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance source (a manufacturer) was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity/grade of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using concurrent negative (vehicle-only) controls. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study type (the detection of adducts is indicative of a positive result). | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance was inferred to be dissolved in vehicle. "Sample preparation" was cited to another publication. Storage was not reported, but not likely to impact the study results (owing to the short-term nature of the study). | |
| Metric 8: | Consistency of Exposure Administration | Not Rated | NA | NA | Animal treatment was cited to other publications. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported clearly. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration (i.e., single gavage dose) were reported and adequate for the study type. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | The number of exposure groups (2 doses plus controls) was reported and considered adequate to address the purpose of the study. Although doses were justified based on previous studies, toxicity (evidenced by serum enzyme activities) was evident at both doses. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The exposure route (intraperitoneal injection) is suited to the test substance but not environmentally relevant. | |
| Domain 4: Test Organism | | | | | | |
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Study Citation: M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plataras, Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710

Data Type: DNA adducts for CCl4

HERO ID: 194788

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

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

Data Type: DNA adducts for CCl4

HERO ID: 194788

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

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

| Study Citation: | M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plataras, Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | DNA damage for CCl4 | | | | | |
| HERO ID: | 194788 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified by name. | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | The source of the test substance source (a manufacturer) was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition. | |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | The purity/grade of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using concurrent negative (vehicle-only) controls. | |
| | Metric 5: Positive Controls | Not Rated | NA | NA | This metric is not applicable for the study type. | |
| | Metric 6: Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance was inferred to be dissolved in vehicle. "Sample preparation" was cited to another publication. Storage was not reported, but not likely to impact the study results (owing to the short-term nature of the study). | |
| | Metric 8: Consistency of Exposure Administration | Not Rated | NA | NA | Animal treatment was cited to other publications. | |
| | Metric 9: Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported clearly. | |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration (i.e., single gavage dose) were reported and adequate for the study type. | |
| | Metric 11: Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The number of exposure groups (2 doses plus controls) was reported and considered adequate to address the purpose of the study. Although doses were justified based on previous studies, toxicity (evidenced by serum enzyme activities) was evident at both doses. | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | The exposure route (intraperitoneal injection) is suited to the test substance but not environmentally relevant. | |
| Domain 4: Test Organism | | | | | | |
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Study Citation: M. B. Kadiiska, B. C. Gladen, D. D. Baird, D. Germolec, L. B. Graham, C. E. Parker, A. Nyska, J. T. Wachsman, B. N. Ames, S. Basu, N. Brot, G. A. Fitzgerald, R. A. Floyd, M. George, J. W. Heinecke, G. E. Hatch, K. Hensley, J. A. Lawson, L. J. Marnett, J. D. Morrow, D. M. Murray, J. Plataras, Roberts LJ 2nd, J. Rokach, M. K. Shigenaga, R. S. Sohal, J. Sun, R. R. Tice, D. H. Van Thiel, D. Wellner, P. B. Walter, K. B. Tomer, R. P. Mason, J. C. Barrett (2005). Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine, 38(6,6), 698-710

Data Type: DNA damage for CCl4

HERO ID: 194788

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|-----------------------|------|------------|---|
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Test animal characteristics were partially reported (obtained from a commercial source, sex, strain, range of body weights). Minor uncertainties in the reporting of test animal characteristics (e.g., health status and age) are unlikely to have a substantial impact on results. |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Animal husbandry conditions (temperature, humidity, light/dark) were reported and appropriate for the study. |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per group (5 males) was reported and adequate for analysis. |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment methodology was cited to another publication; little information was provided in the study. It is not clear that methods were sensitive for the outcome since effects were only observed at the lowest tested dose. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable for the study type. |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | Blinding was reported. The study indicated that samples were marked with a code number so that those conducting assays were unaware of treatment status. |
| | Metric 20: Negative Control Response | Unacceptable | × 1 | 4 | The biological responses of the negative control groups were not reported. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variables in test design or procedures were reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | No health outcomes unrelated to exposure were reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was reported and appropriate for the data set. |
| | Metric 24: Reporting of Data | Unacceptable | × 2 | 8 | Data presentation was inadequate. Although study results (and their statistical significance) were discussed in the text (roughly by exposure group and time point), data were not quantified; the information reported was not sufficient for an independent interpretation of the study results (particularly the positive result seen only at the low dose at one time point). |
| Overall Quality Determination[‡] | | Unacceptable** | | 2.0 | |

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

Data Type: DNA damage for CCl4
HERO ID: 194788

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | Yes | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 89: Animal toxicity evaluation results of Diaz Gomez et al., 1980 study on binding to rat and mouse liver DNA

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: M. I. Diaz Gomez, J. A. Castro (1980). Covalent binding of carbon tetrachloride metabolites to liver nuclear DNA, proteins, and lipids Toxicology and Applied Pharmacology, 56(2,2), 199-206 | | | | | |
| Data Type: Binding to rat and mouse liver DNA for CCl4 | | | | | |
| HERO ID: 194790 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Manufacturer of radiolabeled test substance was reported. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | It was indicated that the test substance was 99% pure. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Negative controls were not needed for the primary DNA binding experiment. Vehicle controls were used for some supplemental experiments evaluating changes in response to a tracer dose of radiolabeled CCl4 (Table 2). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation in olive oil was described; storage was not reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure appeared consistent across groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Specific activity was provided for radiolabeled substances. The toxic dose of CCl4 could be calculated from the information provided. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration were reported (single injection dose). |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | The use of a single dose is adequate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Intraperitoneal injection in olive oil is appropriate for the test substance, but this is not an environmentally relevant route. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source of the test animal was not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported. |
| Metric 15: | Number per Group | Medium | × 1 | 2 | 3 rats/group; 30 mice/group (livers from 10 mice were pooled for each sample) |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Methods were partially described and cited to several other publications. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across groups. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable for the outcome of interest. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable for the outcome of interest. |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | Negative controls were not necessary for the primary experiment. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food, and water intake, were not reported for each treatment group. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The statistical methods were described and appropriate. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were presented for all outcomes. |
| Overall Quality Determination [‡] | | Medium | | 1.8 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 90: Animal toxicity evaluation results of Draper et al., 1995 study on deoxyguanine-MDA adducts

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: H. H. Draper, S. Agarwal, D. E. Nelson, J. J. Wee, A. K. Ghoshal, E. Farber (1995). Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA Lipids, 30(10,10), 959-961 | | | | | |
| Data Type: Deoxyguanine-MDA adducts for CCl4 | | | | | |
| HERO ID: 194814 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Source was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of test substance were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | In Table 1, the study authors indicated the use of a concurrent negative control group, but details regarding the negative control group were not reported. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | CCl4 was apparently administered neat by oral gavage (10 uL). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Gavage volume was not excessive. |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Dose can be calculated from the gavage volume assuming 100 g bw (approximate weight specified in the methods for the CCl4 experiment). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single dose is adequate for the outcome of interest. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single dose level was not justified. |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | Oral gavage is an appropriate route, but a vehicle should be used to avoid damage to the gastrointestinal tract (unclear that a vehicle was used). |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source, strain, and sex of test animals were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | The number of animals used was reported (5/group) and is adequate for the outcome of interest. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Not Rated | NA | NA | The outcome assessment methods were cited to Agarwal and Draper (1992). |
| Metric 17: | Consistency of Outcome Assessment | Not Rated | NA | NA | The outcome assessment methods were cited to Agarwal and Draper (1992). |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|--|
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric does not apply to the outcome of interest. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric does not apply to the outcome of interest. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response appears adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food and water intake were not reported across groups. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Low | × 1 | 3 | Statistics were performed, but the method was not reported. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported as mean +/-SEM. |
| Overall Quality Determination [‡] | | Low | | 2.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 91: Animal toxicity evaluation results of Rocchi et al., 1973 study on DNA binding

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| Study Citation: P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425 | | | | | |
| Data Type: DNA binding for CCl4 | | | | | |
| HERO ID: 194878 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as radiolabeled carbon tetrachloride (CCl4-14C). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the radiolabeled test substance was reported. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The specific activity of the CCl4-14C was reported. The test substance purity was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Negative control groups were not included (and not necessarily required by the study type). It was noted that the counts per minute (cpm) of samples were at least twice background levels. However, controls were not mentioned, and it was not indicated how samples were corrected for radioactivity levels. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study type. The identification of labeled DNA was evidence of a positive result. Methylcholanthracene pre-treatment was used to stimulate hepatic metabolism. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Details regarding preparation of the test substance were limited (dissolved in corn oil). Storage of the test substance was not reported, but this is appropriate given the study design (single-dose administration). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was appropriate and consistent among study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | The dose used in the study was reported (326 UCi corresponding to 367 umol/kg). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency/duration (single injection) were reported and appropriate for the study design. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Only one dose level was utilized, but this is considered to be adequate given the study design. A rationale for the selection of this dose level was not provided. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The route of exposure was reported and appropriate (but not environmentally relevant). |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The source of the test animals were not reported. The species, strain, life stage (adult), sex, and point estimate of body weight were reported. |

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| Study Citation: | P. Rocchi, G. Prodi, S. Grilli, A. M. Ferreri (1973). In vivo and in vitro binding of carbon tetrachloride with nucleic acids and proteins in rats and mouse liver International Journal of Cancer, 11(2,2), 419-425 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA binding for CCl4 | | | | | |
| HERO ID: | 194878 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not reported. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | There were 3 rats per treatment group and 25 mice per treatment group; however livers were pooled by treatment group. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodology was partially described (e.g., radioactivity measurement) and partially cited to other sources (isolation of DNA, RNA, and protein fractions). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was assessed consistently among treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | This metric is not applicable to the study design, as no negative controls were utilized. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | No confounding variables unrelated to exposure were identified. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Since livers were pooled by treatment group, no standard deviations were reported, and statistical analysis was not possible. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Data were reported by group (pre-treated or not; without control data or a measure of variation). | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.2 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[\frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 92: Animal toxicity evaluation results of Gans et al., 1984 study on DNA damage

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

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| Study Citation: | J. H. Gans, R. Korson (1984). Liver nuclear DNA synthesis in mice following carbon tetrachloride administration or partial hepatectomy Proceedings of the Society for Experimental Biology and Medicine, 175(2,2), 237-242 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA damage for CCl4 | | | | | |
| HERO ID: | 194904 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and weight (range) were reported. The commercial source was reported (Charles River); health status and age were not reported. The test species and strain were an appropriate animal model for the evaluation of this endpoint. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Husbandry conditions were partially described with some omissions (temperature and humidity). | |
| | Metric 15: Number per Group | Unacceptable | × 1 | 4 | The number of animals per group was not reported; it was noted that there were 9 mice in the control group. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodologies were partially described; the methodology was further described in previously published papers (Cox et al., 1973 and Laishes et al., 1975) | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | The outcome assessment methodologies were described in previously published papers (Cox et al., 1973 and Laishes et al., 1975), but some details were briefly described and appeared consistent. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for this study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for this study type. | |
| | Metric 20: Negative Control Response | Low | × 1 | 3 | The control response was reported; the responses appear adequate; however, there is some uncertainty in the reporting (only limits of sedimentation were reported graphically). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions for each study group is not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Statistical analysis between treated and control groups was not conducted for the alkaline elution study. Data are not amenable to independent analysis. | |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Data for exposure-related findings were not shown for each study group; data were only reported for some groups/outcomes. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.3 | | |
| Extracted | | No | | | | |
| Continued on next page ... | | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 93: Animal toxicity evaluation results of Sarkar et al., 1999 study on chromosomal aberrations

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|--|---------------------|------|-------|---|
| Study Citation: A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by <i>Trianthema portulacastrum</i> L. in carbon tetrachloride-induced mouse liver damage <i>Cell Biology International</i> , 23(10,10), 703-708 | | | | | |
| Data Type: Chromosomal aberrations for CCl4 | | | | | |
| HERO ID: 194915 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride (CCl4). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The grade/purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using a concurrent negative (vehicle-only) control. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control was not used; however, positive responses were observed in this study (i.e., demonstrating the test is capable of detecting a positive response). |
| Metric 6: | Randomized Allocation | High | × 1 | 1 | The study indicated that animals were randomly allocated. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | It was indicated that the test substance was dissolved in olive oil; however, storage was not reported. |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Gavage volumes were not consistently reported. Based on the information provided, it appears that exposures were applied consistently (7 weeks daily distilled water plus vehicle-only or CCl4 exposure three times weekly for 5 weeks). |
| Metric 9: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | The dose was reported as 20%/mouse; the dose in mg/kg/day cannot be determined. |
| Metric 10: | Exposure Frequency and Duration | Medium | × 1 | 2 | Frequency/duration of exposure was reported (3 times/week for 5 weeks). The applicability of a repeated-dose protocol to this study type is uncertain. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | The study utilized one dose of CCl4; the abstract indicated that the dose was necrogenic. Although no rationale was provided, the study authors indicated that the dose used was adequate to increase the percentage of aberrant metaphase cells. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The exposure route/method was reported and appropriate (i.e., oral gavage). |
| Domain 4: Test Organism | | | | | |
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| Study Citation: | A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by <i>Trianthema portulacastrum</i> L. in carbon tetrachloride-induced mouse liver damage <i>Cell Biology International</i> , 23(10,10), 703-708 | | | | | |
|---|---|---------------------|------|-------|--|--|
| Data Type: | Chromosomal aberrations for CCl4 | | | | | |
| HERO ID: | 194915 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Minor uncertainties in the reporting of test animal characteristics (e.g., health status, age) are unlikely to have a substantial impact on results. The test animals were obtained from a commercial source. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | Husbandry conditions were reported (temperature, humidity, light-dark cycle). | |
| | Metric 15: Number per Group | High | × 1 | 1 | The study indicated that there were 15-22 mice/group; based on Table 1 (chromosomal aberrations), data were for 5-7 mice/time point (15, 30, or 45 days after exposure). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment methodology was partially described and partially cited to other publications (e.g., chromosome preparation cited to Horiuchi et al., 1984). In addition, it is not clear that the reported outcome assessment methodology was appropriate for the outcome of interest (e.g., evaluation of major structural chromosomal aberrations 15, 30, and 45 days after the last CCl4 dose). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Based on data in Table 1, major chromosomal aberrations were scored in each group at the same time points after treatment. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | The study indicated that 50 metaphases were analyzed by animal (or about 250/group); this is fewer than recommended for studies of this type. | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The study indicated that, for chromosomal aberrations, coded slides were scored blind. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The study authors indicated that the response of the negative control group was adequate (e.g., low numbers of chromosomal aberrations). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information regarding initial animal conditions were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | The study indicated that "data were analyzed statistically for differences between the mean using Student's t-test" and that significance was established at $p < 0.05$. However, it does not appear that comparisons were made between controls and CCl4-treated groups, and data are not provided (by time point) for independent analyses. | |
| Continued on next page ... | | | | | | |

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| Study Citation: | A. Sarkar, S. Pradhan, I. Mukhopadhyay, S. K. Bose, S. Roy, M. Chatterjee (1999). Inhibition of early DNA-damage and chromosomal aberrations by <i>Trianthema portulacastrum</i> L. in carbon tetrachloride-induced mouse liver damage <i>Cell Biology International</i> , 23(10,10), 703-708 | | | | |
|--|---|---------------------|------|-------|--|
| Data Type: | Chromosomal aberrations for CCl4 | | | | |
| HERO ID: | 194915 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| | Metric 24: Reporting of Data | Unacceptable | × 2 | 8 | Data for CCl4 treatment groups are provided by time point; however, the table indicates only the mean value for the control group (overall). |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.2 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 94: Animal toxicity evaluation results of Sawada et al., 1991 study on rat liver chromosome aberration, micronuclei, SCE

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: S. Sawada, T. Yamanaka, K. Yamatsu, C. Furihata, T. Matsushima (1991). Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced in vivo by hepatocarcinogens including heterocyclic amines Mutation Research, 251(1,1), 59-69 | | | | | |
| Data Type: Rat liver chromosome aberration, micronuclei, SCE | | | | | |
| HERO ID: 194926 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified by chemical name, formula and CASRN. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of the test substance was Wako Pure Industries, Osaka. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity or grade was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Vehicle control group was used for a negative control. |
| Metric 5: | Positive Controls | High | × 1 | 1 | Positive responses were elicited with other substances tested including DMN and 2-AAF. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was prepared in corn oil; however, storage conditions or solution are not reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was consistent and appropriate across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Dose was reported without ambiguity (1600mg/kg). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were reported and appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Only one concentration of test substance was studied, which produced negative results. It is unclear if a higher dose would have elicited positive responses. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route was appropriate. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Information on test animals was provided, purchased from Charles River Japan, Kanagawa. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | High | × 1 | 1 | 3-4 rats per time point were studied. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Methodology is partially described and cited elsewhere. |
| Metric 17: | Consistency of Outcome Assessment | Medium | × 1 | 2 | Minor details regarding outcome assessment protocol are not provided, this is unlikely to have a substantial impact on results. |
| Metric 18: | Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for outcome of interest. |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | Blinding is not applicable to this study. |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control response was adequate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions of test animals were not fully reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on health status after treatment are not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis performed was appropriate (t-test). |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for all outcomes were reported. |
| Overall Quality Determination [‡] | | High | | 1.6 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 95: Animal toxicity evaluation results of Brambilla et al., 1983 study on fragmentation of liver DNA

| Study Citation: | G. Brambilla, P. Carlo, R. Finollo, F. A. Bignone, A. Ledda, E. Cajelli (1983). Viscometric detection of liver DNA fragmentation in rats treated with minimal doses of chemical carcinogens Cancer Research, 43(1,1), 202-209 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Fragmentation of liver DNA | | | | | |
| HERO ID: | 194933 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of test substance was E. Merck (Darmstadt, West Germany) | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity or grade was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative controls rats were administered the vehicle. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls were not employed, however positive responses were elicited with other test substances. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Unacceptable | × 1 | 4 | Information on how test substance was prepared was not reported. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was administered consistently across study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Dose was reported as 200 mg/kg. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were reported and appropriate. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Only one dose was studied which resulted in a negative response. It is unclear if higher doses would have elicited a response. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route was appropriate. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | Source of the animals was not reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. | |
| Metric 15: | Number per Group | Medium | × 1 | 2 | Three rats / groups were studied. | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome assessment methodology was partially described and cited elsewhere. | |
| Metric 17: | Consistency of Outcome Assessment | Medium | × 1 | 2 | There were incomplete reporting of outcome assessment methodology. | |
| Metric 18: | Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for outcome of interest. | |
| Metric 19: | Blinding of Assessors | Not Rated | NA | NA | Blinding was not applicable to this study. | |
| Metric 20: | Negative Control Response | High | × 1 | 1 | Negative control response was adequate. | |

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

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 96: Animal toxicity evaluation results of Levy et al., 1984 study on mitochondrial and nuclear DNA binding in rat liver

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234 | | | | | |
| Data Type: mitochondrial and nuclear DNA binding in rat liver | | | | | |
| HERO ID: 194952 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (CCl ₄). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was identified (Amersham). The product number and batch/lot number were not reported; however the material is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of the test substance was reported (>99%). |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | The use of a negative control group is not necessary for DNA binding assays; measurement of radiolabeled test compound is the outcome. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive control was used; however, is not necessary for this study type. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Animal allocation methodology was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance preparation was reported. The storage of the test substance was not reported; however, it was a single administration (unlikely to affect results). |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration were reported and appear to be administered consistently. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Administered dose was reported. (2 µl/kg = 2.1 x 10 ⁻² µmol/kg; 1.1 ml/kg = 11.4 µmol/kg) |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency and duration were appropriate for this endpoint; single dose |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | 2 doses were tested and 2 time points for the low dose group; known non-necrotizing or acutely toxic doses. |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | The route or method of exposure was not reported; it could be oral or some injection route, but the study does not specify. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The test animal species, strain, sex, and weight were reported. Health status and age were not reported. The commercial source was reported (Charles River). The test species and strain were an appropriate animal model for the evaluation of this endpoint. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate. |
| Metric 15: | Number per Group | Unacceptable | × 1 | 4 | The number of animals per study group was not reported |

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| Study Citation: | G. N. Levy, M. J. Brabec (1984). Binding of carbon tetrachloride metabolites to rat hepatic mitochondrial DNA Toxicology Letters, 22(2,2), 229-234 | | | | |
|--|--|---------------------|------|-------|--|
| Data Type: | mitochondrial and nuclear DNA binding in rat liver | | | | |
| HERO ID: | 194952 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Details of the outcome assessment protocol were reported; 2 time points were analyzed for the low dose group (5 and 24 hours), but only analyzed at 1 time point (24 hours) for the high dose group. |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Details of sampling of outcomes were not clearly reported; number of animals tested were not reported; DNA binding results were calculated based on 3.237 umol/mg DNA |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for this study type |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | The use of a negative control group is not necessary for DNA binding assays |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight was reported, though food and water intake were not reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analysis was not conducted. DNA binding data were provided as a mean and standard deviations for a specified number of experiments; independent statistical analysis may be performed. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for exposure-related findings were presented for dose groups, preparations, and time points with quantal presentation. |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.8 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 97: Animal toxicity evaluation results of Cabra et al., 1999 study on DNA damage

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: M. Cabré, N. Ferré, J. Folch, J. L. Paternain, M. Hernández, D. Del Castillo, J. Joven, J. Camps (1999). Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats <i>Journal of Hepatology</i> , 31(2,2), 228-234 | | | | | |
| Data Type: DNA damage for CCl4 | | | | | |
| HERO ID: 194968 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The test substance source was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative controls received vehicle injection (olive oil). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The method of animal allocation was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance (1:1 dilution in olive oil) was reported. Storage of the test substance between injections was not reported. This deficiency is not expected to have substantially impacted results. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | The dose of CCl4 was described as 0.5 mL CCl4 per kg body weight. This was calculated by the reviewer to be 795 mg/kg based on the density of CCl4 (1.59 g/mL). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency (two i.p. injections 4 days apart) and duration (sacrificed 1 day after second injection) was considered to be appropriate. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Only one dose level was utilized. The dose level was considered to be adequate, as positive results were observed. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route of exposure was appropriate for the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | Test animal species, strain, sex, and starting body weight range were reported. Test animal health status and age were not reported. This is not expected to have substantially impacted results. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | It was described that animals were on a reverse 12-hour light cycle. The justification for this reverse light cycle was not included. The temperature and humidity of the animal rooms were not reported. These deficiencies are not expected to have substantially impacted results. |

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| Study Citation: | M. Cabré, N. Ferré, J. Folch, J. L. Paternain, M. Hernández, D. Del Castillo, J. Joven, J. Camps (1999). Inhibition of hepatic cell nuclear DNA fragmentation by zinc in carbon tetrachloride-treated rats <i>Journal of Hepatology</i> , 31(2,2), 228-234 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA damage for CCl4 | | | | | |
| HERO ID: | 194968 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The control group had n = 11 rats and the CCl4-only group had n = 12 rats. These numbers are considered adequate for the study design. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate to address the intended outcome (DNA fragmentation). The TUNEL assay is usually utilized to assess DNA fragmentation as a result of apoptosis, but DNA strand breaks from other causes will be detected as well by this assay. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was assessed consistently across treatment groups. | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | It was noted that 25,000-30,000 cells per slide were counted. It was not clear how many slides per animal were included. | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | It was noted that the identity of the slides was coded prior to analysis. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative controls responded appropriately. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Information on initial conditions for each study group is not reported (body weight, food, water intake). | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Data were analyzed appropriately. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were reported adequately. | |
| Overall Quality Determination [‡] | | High | | 1.6 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 98: Animal toxicity evaluation results of Castro et al., 1989 study on DNA and nuclear protein binding in vivo

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|--|
| Study Citation: G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294 | | | | | |
| Data Type: DNA and nuclear protein binding in vivo | | | | | |
| HERO ID: 194983 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as CCL4, CASRN provided. Radiolabeled CCL4 also used. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Commercial sources were reported. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Unlabeled CCL4 reported to be "low sulfur quality", - labeled CCL4 purity 99% |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | Not applicable for the study design |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Not applicable for the study design |
| Metric 6: | Randomized Allocation | Not Rated | NA | NA | Not applicable, only a single group of animals were used and random allocation is not necessary. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Storage of radiolabeled CCL4 was reported and stability was tested/confirmed. Test substance was prepared as a 15% v/v solution in olive oil prior to administration. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure were reported, and volume was appropriate (5ml/kg, ip) |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | Dose was not reported in mg/kg bw, but as 1.1 x 10 ⁻⁸ d.p.m/mL solution administered at a dose of 5 mL/kg. Animal body weight ranges were provided. Justification for the chosen dose was not provided. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure and duration were appropriate for the outcome of interest. Single injection, animals were sacrificed after 6 hours |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | A single exposure group was acceptable and appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The exposure route (i.p) was acceptable for the test substance and for the outcome of interest. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | Three species (rat, mouse, hamster) were included. Strains, sex, and body weight ranges were provided. The sources of the animals were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not reported. |

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| Study Citation: | G. D. Castro, M. I. Diaz Gomez, J. A. Castro (1989). Species differences in the interaction between CCl4 reactive metabolites and liver DNA or nuclear protein fractions Carcinogenesis, 10(2,2), 289-294 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | DNA and nuclear protein binding in vivo | | | | | |
| HERO ID: | 194983 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per group was appropriate. Triplicate experiments were done on pooled samples from 6 mice, 2 hamsters, and 1 rat per sample. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Outcome assessment methodology was appropriate for the outcome of interest. Some details were described, some methods were cited to another publication. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Consistency in outcome assessment between replicates was assumed from the text. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling adequacy was appropriate for the outcome of interest (sufficient concentrations of covalently bound DNA and protein to accurately detect above background) | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable for the outcome of interest. | |
| | Metric 20: Negative Control Response | Not Rated | NA | NA | Not applicable (no negative control) | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Confounding variables in the test design and procedure were not reported. Since only one group of animals were tested, there were no concerns about potential differences between groups | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Health outcomes unrelated to exposure (e.g., animal health prior to the start of the study) were not reported. Since only one group of animals were tested, there were no concerns about potential differences between groups | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis to evaluate differences between species was performed and appropriate (student T-test). Data were presented as Means with SD | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for all of the outcomes of interest were adequately reported | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: V. M. Craddock, A. R. Henderson (1978). De novo and repair replication of DNA in liver of carcinogen-treated animals Cancer Research, 38(7,7), 2135-2143 | | | | | |
| Data Type: Unscheduled DNA synthesis in rat liver after i.p. exposure | | | | | |
| HERO ID: 195014 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of test substance was Fisons Scientific Apparatus Ltd. (Loughborough, Leicestershire, England.) |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity or grade of test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Unacceptable | × 2 | 8 | A concurrent negative control was not used. Untreated rat data came from a previously published study (Craddock, 1976). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive control was not employed, however positive responses were elicited with other test substances. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Method of allocation of animals was not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was mixed with equal volume of liquid paraffin, however concentration of prepared mixture (mg/ml) was not reported nor were storage conditions. Storage conditions unlikely to substantially impact results of this single exposure study. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was administered consistently, and gavage volume was appropriate (reported as 2.5 ml/kg in Chart 4 ; CCL4 was prepared in an equal volume of liquid paraffin yielding gavage volume of 5 ml/kg) |
| Metric 9: | Reporting of Doses/Concentrations | Medium | × 2 | 4 | Dose is reported as 2.5 ml/kg in Chart 4 or 4000 mg/kg in Table 1 (doses equivalent based on density of 1.59 g/ml). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/ duration (once) were reported and appropriate for this study. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Dose used was justified as previously shown to cause necrosis at later timepoints. In the absence of a negative control it is not possible to determine whether the dose was appropriate. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route (gavage) was appropriate. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Low | × 2 | 6 | The species, strain, sex, and initial body weight of test animals were reported. The source of test animals was not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |

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| Study Citation: | V. M. Craddock, A. R. Henderson (1978). De novo and repair replication of DNA in liver of carcinogen-treated animals <i>Cancer Research</i> , 38(7,7), 2135-2143 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Unscheduled DNA synthesis in rat liver after i.p. exposure | | | | | |
| HERO ID: | 195014 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | 4 animals/groups were studied. This is lower than the typical number (5/group) for this type of study. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Methodology is partially described and cited elsewhere. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Incomplete reporting of outcome assessment protocol; however limitations are unlikely to have substantial impact on results. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling was adequate for outcome of interest (4 g liver from each animal pooled for nuclei isolation) | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding not applicable to this study. | |
| | Metric 20: Negative Control Response | Low | × 1 | 3 | The biological response of negative control group was adequate; however, data were obtained from previous study (Craddock, 1976) rather than concurrent with this experiment | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions (food/water intake, health condition) of rats are not fully reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition or health outcomes unrelated to exposure are not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | Statistical analysis is not necessary for this outcome | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Results for exposed groups are reported. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.1 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 100: Animal toxicity evaluation results of Nakamura et al., 1992 study on DNA strand breaks for CCl4 (acute and chronic)

| Study Citation: | T. Nakamura, M. Hotchi (1992). Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury Virchows Archiv B Cell Pathology Including Molecular Pathology, 63(1,1), 11-16 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | DNA strand breaks for CCl4 (acute and chronic) | | | | | |
| HERO ID: | 195152 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride (CCl4). | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance (a manufacturer) was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Results section compares data to “untreated controls”. Details pertaining to the control are not provided. The study does indicate that for in situ nick translation (ISNT), sections treated without DNA polymerase (unstained) were used as a negative control. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A positive control was not employed (or required by study type). | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Random allocation of animals was not reported. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of test substance was provided (dissolved in olive oil); however, storage conditions were not reported. | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across/within study groups. | |
| Metric 9: | Reporting of Doses/Concentrations | Low | × 2 | 6 | Dose concentrations were reported as 20% CCl4 administered at 5mL/kg (acute study) and 50% CCl4 administered at 2.5mL/kg (“chronic” study). Doses can be estimated based on (initial) approximate body weight provided (150 g); however, body weights would be expected to change over the course of the chronic study (12 weeks). | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Frequency/duration of exposures were reported (once for the “acute” study, and twice weekly for up to 12 weeks for the “chronic” study). | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | The number of exposure groups was reported (1 dose group for each study, evaluated over multiple time points). A rationale for the selected doses was not provided (presumably based on previous studies). | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Route of exposure was reported (intraperitoneal or subcutaneous injection), but not environmentally relevant. | |
| Domain 4: Test Organism | | | | | | |

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| Study Citation: | T. Nakamura, M. Hotchi (1992). Changes in DNA strand breaks in non-parenchymal cells following hepatocyte regeneration in CCl4-induced rat liver injury Virchows Archiv B Cell Pathology Including Molecular Pathology, 63(1,1), 11-16 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA strand breaks for CCl4 (acute and chronic) | | | | | |
| HERO ID: | 195152 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | High | × 2 | 2 | Pertinent information on test animal was reported (source, strain, sex, age, initial body weights). | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Based on information provided in the methods section with respect to the number of animals subjected to acute and chronic exposure and the time points of sacrifice, there were likely 3-4/rats/time point (fewer than recommended by study type). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | The outcome assessment methodology is described (original source of the method is also cited). It is noted that the methodology was applied to evaluate DNA strand breaks as a marker for proliferation, differentiation, and/or activated gene expression (rather than DNA injury). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | It appears that outcomes were assessed consistently in the study groups. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | Details on sampling outcomes were limited. The legend to Figure 2 describes data as the number of positive cells/10 high power fields (presumably expressed as a mean for animals evaluated, but not specified). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| | Metric 20: Negative Control Response | Medium | × 1 | 2 | Some data for untreated animals were described qualitatively on the text; data for the 0 hour/week time point were shown in Figure 2. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes of study groups (unrelated to exposure) were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Unacceptable | × 1 | 4 | Statistical analyses were not described. The data presented in Figure 2 are not amenable to independent analyses because the "n" and measure of variance (SD or SEM) shown are not clearly reported. | |
| | Metric 24: Reporting of Data | | × 2 | NA | Data for each study (acute and chronic) by time point are presented in Figure 2. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 0.0 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 101: Animal toxicity evaluation results of L³pez-Diazguerrero et al., 2005 study on comparison of oxidative DNA damage in young and old mice

| Study Citation: | N. E. López-Diazguerrero, A. Luna-López, M. C. Gutiérrez-Ruiz, A. Zentella, M. Königsberg (2005). Susceptibility of DNA to oxidative stressors in young and aging mice <i>Life Sciences</i> , 77(22,22), 2840-2854 | | | | |
|-------------------------------------|--|---------------------|------|-------|---|
| Data Type: | Comparison of oxidative DNA damage in young and old mice | | | | |
| HERO ID: | 195160 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of test substance was identified as Sigma (ST. Louis, MO) |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance was reported to be of the highest analytical grade available. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative control group was treated concurrently with vehicle. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Treatment related positive responses were elicited. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Method of allocation of animals is not reported. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of test substance is described; however, storage conditions are not. As the study was only 3 days in duration, the lack of storage information is unlikely to significantly impact the results. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Animals were exposed consistently across study groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Dose was reported as 0.16 ml/kg, |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency (once per day) and duration (3 days) were reported. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Only one dose was used; it was not justified by the authors, but was sufficient to yield a positive response. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route and method (i.p. injection) were appropriate. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Species, strain, sex, age, and source were reported. Test animals were obtained from a closed breeding colony from Universidad Autonoma Metropolitana-Iztapalapa. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were reported to be in accordance with Mexican official ethics standard; no further details provided |
| Metric 15: | Number per Group | Medium | × 1 | 2 | 3 animals/group were used; this is a smaller size than typical for this outcome but sufficient for statistical analysis |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | Outcome assessment methodology was reported fully and sensitive for the outcome of interest. |

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| Study Citation: | N. E. López-Diazguerrero, A. Luna-López, M. C. Gutiérrez-Ruiz, A. Zentella, M. Königsberg (2005). Susceptibility of DNA to oxidative stressors in young and aging mice <i>Life Sciences</i> , 77(22,22), 2840-2854 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Comparison of oxidative DNA damage in young and old mice | | | | | |
| HERO ID: | 195160 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was carried out consistently across study groups. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling is adequate for study design (100 ug samples DNA analyzed for 8-oxodGuo) | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not necessary. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Response of negative controls was reported and appeared to be appropriate. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial condition (body weight, food/water intake, health status) of animals is not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical analysis was described and appropriate (Student's t test.) | |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Data reported graphically for all groups. Legend of figure 1 states that data shown are mean ±SD of at least three independent experiments. | |
| Overall Quality Determination [‡] | | High | | 1.5 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 102: **Animal toxicity evaluation results of Kitta et al., 1982 study on DNA damage in rat liver after i.p. exposure**

| Study Citation: | D. Kitta, M. Schwarz, H. A. Tennekes, H. Uehleke, W. Kunz (1982). Covalent binding of CCl4-intermediates to reduced pyridine nucleotides in mouse liver <i>Advances in Experimental Medicine and Biology</i> , 136 769-777 | | | | |
|--|--|---------------------|------|-------|---|
| Data Type: | DNA damage in rat liver after i.p. exposure | | | | |
| HERO ID: | 195226 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified by name and molecular formula |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Test substance source was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Study authors reported concurrent negative control but it is not clear whether it was untreated or sham/vehicle treated |
| Metric 5: | Positive Controls | Unacceptable | × 1 | 4 | Positive control was not used and results generally negative. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The method of animal allocation was not reported |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Unacceptable | × 1 | 4 | Test substance preparation and storage were not reported. It is not clear whether a vehicle was used. |
| Metric 8: | Consistency of Exposure Administration | Not Rated | NA | NA | Available information was not sufficient to determine whether exposures were administered consistently. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Dose was reported clearly as 4 mg CCL4/kg bw |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single dose study, appropriate to outcome of interest. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Single dose group included; dose was not justified by study authors, and results were negative |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | i.p administration was used and was not justified by study authors. I.p. administration is generally not recommended for this study type. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Unacceptable | × 2 | 8 | Strain, sex, age, body weight, and source of test animal were not reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate |
| Metric 15: | Number per Group | Unacceptable | × 1 | 4 | Number of animals per group was not reported |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment methods cited to another publication with no additional details. |
| Metric 17: | Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment methods cited to another publication with no additional details. |
| Metric 18: | Sampling Adequacy | Low | × 1 | 3 | Details regarding outcome sampling were not reported. |

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| Study Citation: | D. Kitta, M. Schwarz, H. A. Tennekes, H. Uehleke, W. Kunz (1982). Covalent binding of CCl4-intermediates to reduced pyridine nucleotides in mouse liver <i>Advances in Experimental Medicine and Biology</i> , 136 769-777 | | | | |
|--|--|---------------------|------|-------|---|
| Data Type: | DNA damage in rat liver after i.p. exposure | | | | |
| HERO ID: | 195226 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding of assessors was not reported. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The response of negative controls was appropriate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food/water intake were not reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | Statistical analysis was not reported but may not be necessary for interpretation |
| | Metric 24: Reporting of Data | Low | × 2 | 6 | Results reported graphically without measure of variability |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.7 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 103: **Animal toxicity evaluation results of Kitchin and Brown 1989 for acute hepatic DNA damage in rats**

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| Study Citation: K. T. Kitchin, J. L. Brown (1989). Biochemical effects of three carcinogenic chlorinated methanes in rat liver Teratogenesis, Carcinogenesis, and Mutagenesis, 9(1,1), 61-69 | | | | | |
| Data Type: Acute hepatic DNA damage in rats for CCl4 | | | | | |
| HERO ID: 195230 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride (CCl4). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The test substance purity was not reported, but it was noted that it was "ACS grade". |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent solvent control groups were included (corn oil gavage). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | High | × 1 | 1 | Preparation of the test substance was briefly reported. Storage of the test substance was not reported, but this is appropriate given the acute timeframe of the study. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was reported to be consistent across treatment groups. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity. |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | The exposure frequency and duration were reported and appropriate for this endpoint. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Unacceptable | × 1 | 4 | The dose spacing was appropriate. The number of groups was somewhat lacking at 2. Rats were treated with 15, 70, 350, and 1050 mg/kg CCl4, but only the two highest doses were assessed for DNA damage. Furthermore, the two highest doses also showed increased ALT, an indicator of hepatotoxicity. This indicates that these doses caused cytotoxicity in addition to DNA damage, and that the findings of DNA damage at these doses are unreliable. Lower doses below the level that induces hepatotoxicity should have been assessed for DNA damage. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were appropriate for the test substance. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | Medium | × 2 | 4 | The species, strain, sex, age, and commercial source of the test animals were reported. Starting body weights of the test animals were not reported. |
| Continued on next page ... | | | | | |

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| Study Citation: | K. T. Kitchin, J. L. Brown (1989). Biochemical effects of three carcinogenic chlorinated methanes in rat liver Teratogenesis, Carcinogenesis, and Mutagenesis, 9(1,1), 61-69 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Acute hepatic DNA damage in rats for CCl4 | | | | | |
| HERO ID: | 195230 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported other than the number of rats per cage. | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per treatment group was adequate and appropriate for these endpoints (n = 8 for all groups). | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for this endpoint. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment methodology was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Low | × 1 | 3 | It was not clear how many technical replicates per animal were included in the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the outcome of interest. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative responses were observed in negative controls. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Starting body weight ranges were not included. Food and water consumption and respiratory rates were not reported, but this is appropriate given the study design. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | No deaths or adverse health findings unrelated to the test compound were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | The data were analyzed appropriately by Bartlett's test for homogeneity of variance and Dunnett's multiple comparison test. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | The data were reported adequately. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.5 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 104: **Animal toxicity evaluation results of Hachiya et al., 2000 study on mutagenicity in liver of MutaMouse**

| Study Citation: | N. Hachiya, Y. Motohashi (2000). Examination of lacZ mutant induction in the liver and testis of Muta(TM)Mouse following injection of halogenated aliphatic hydrocarbons classified as human carcinogens Industrial Health, 38(2,2), 213-220 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | Mutagenicity in liver of MutaMouse | | | | | |
| HERO ID: | 202845 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | Source of test substance was not reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity or grade of test substance is not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | A negative control was used, however there are minor differences in treatment conditions. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive control was not employed, however positive responses were elicited with other substances concurrently tested. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Method of allocation of animals was not reported. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was dissolved in olive oil however final concentration of prepared solution and storage conditions were not reported. Lack of information on storage unlikely to impact results since this was a single exposure study. | |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Gavage volumes and concentration of prepared test substance were not reported. | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses are provided clearly in Table 1 (700 or 1400 mg/kg). | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/duration (single exposure) were reported and appropriate. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Low | × 1 | 3 | Dose levels were not justified by authors, and results were judged to be negative; it is unclear if a higher dose would have elicited a positive response. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure (oral gavage) route was reported and appropriate. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Test animal species, strain, sex, age, and source were reported. | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Animal husbandry conditions were not reported. | |
| Metric 15: | Number per Group | Low | × 1 | 3 | 2-3 animals/groups were tested. This number is lower than typically used for genotoxicity studies. | |
| Domain 5: Outcome Assessment | | | | | | |
| Metric 16: | Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment methodology was cited elsewhere with virtually no additional details provided. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--|---------------------|------|-------|---|
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment execution was cited elsewhere with no additional details provided. |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | The number of phages scored was adequate but varied by group. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not applicable to this study. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The negative control response was reported appropriate. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food/water intake were not reported. |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Health outcomes unrelated to treatment were not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical analysis by Fisher's exact test was reported; no additional details reported. |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data were presented in Table 1. |
| Overall Quality Determination [‡] | | Medium | | 1.9 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 105: **Animal toxicity evaluation results of Sun et al., 2014 for a study on genomics/metabolomics outcomes**

| Study Citation: | Sun, J; Schmitt, T; Schnackenberg, LK; Pence, L; Ando, Y; Greenhaw, J; Yang, Xi; Slavov, S; Davis, K; Salminen, WF; Mendrick, DL; Beger, RD (2014). Comprehensive analysis of alterations in lipid and bile acid metabolism by carbon tetrachloride using integrated transcriptomics and metabolomics <i>Metabolomics</i> , 10(6), 1293-1304 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | | | | | | |
| HERO ID: | 3487830 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | Commercial source was identified. | |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | Purity not reported. | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | Vehicle (corn oil) controls were used. | |
| | Metric 5: Positive Controls | Not Rated | NA | NA | Positive controls were not used for genomic/metabolomics alterations. | |
| | Metric 6: Randomized Allocation | High | × 1 | 1 | Animals were randomly assigned to each dose group. | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 7: Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation and storage were not described; however, omission of these details are unlikely to have a substantial impact on results (acute exposure). | |
| | Metric 8: Consistency of Exposure Administration | High | × 1 | 1 | Gavage volume was not excessive. | |
| | Metric 9: Reporting of Doses/Concentrations | High | × 2 | 2 | | |
| | Metric 10: Exposure Frequency and Duration | High | × 1 | 1 | Genomic/metabolic data provide mechanistic understanding for liver effects which occur after acute exposure. | |
| | Metric 11: Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Adequate number of dose groups. Dose spacing justified by previous research. | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | | |
| Domain 4: Test Organism | | | | | | |
| | Metric 13: Test Animal Characteristics | High | × 2 | 2 | FDA colony; species, strain and starting age reported. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | High | × 1 | 1 | | |
| | Metric 15: Number per Group | High | × 1 | 1 | 15/group | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | Mechanistic changes related to liver toxicity | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | | |
| | Metric 18: Sampling Adequacy | Medium | × 1 | 2 | Summary data for metabolomics and genomics is provided for the high dose group only. Supplemental data tables are available for purchase. | |
| Continued on next page ... | | | | | | |

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

Data Type:
HERO ID: 3487830

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--|--|---------------------|------------------|-------|--|
| | Metric 19: Blinding of Assessors | Medium | × 1 | 2 | Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results. |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Metabolomics changes were reported relative to control. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | Lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results. |
| | Metric 22: Health Outcomes Unrelated to Exposure | High | × 1 | 1 | |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | |
| | Metric 24: Reporting of Data | Medium | × 2 | 4 | Summary data is reported in the paper; supplemental data table are available for purchase. |
| Overall Quality Determination [‡] | | High | | 1.3 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 106: Animal toxicity evaluation results of Sasaki et al., 1998 study on DNA damage (comet assay) in multiple organs of mice exposed orally

| Study Citation: | Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y. Q. Su, N. Matsusaka, S. Tsuda (1998). Detection in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research, 419(1-3,1-3), 13-20 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | DNA damage (comet assay) in multiple organs of mice exposed orally | | | | | |
| HERO ID: | 5447470 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of test substance was Wako (Osaka, Japan). | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity or grade of test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Untreated mice were used as negative control instead of a gavage vehicle control. Authors note that their previous studies showed no difference in response between untreated and vehicle control groups. It is therefore, unlikely to have a substantial impact on results. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive controls were not administered, however positive responses were elicited with other substances. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | Method of allocation of animals was not reported. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Test substance was prepared in olive oil, however final concentration of prepared substance was not reported, nor are the storage conditions. | |
| Metric 8: | Consistency of Exposure Administration | Medium | × 1 | 2 | Gavage volume is not reported. Furthermore, since concentration of prepared substance was not reported, the gavage volume cannot be inferred. Controls were not treated; however, the authors provided evidence that sham treatment would not alter the outcome | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported (500, 1000, or 2000 mg/kg) without ambiguity. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Exposure frequency/ duration (single exposure) were reported and appropriate. | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | High | × 1 | 1 | Maximum dose used was justified based on previous study (Sasaki et al., 1997). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure route (oral) was reported and appropriate; while not specified, it is inferred from the study that the method was gavage. | |
| Domain 4: Test Organism | | | | | | |
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| Study Citation: | Y. F. Sasaki, A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y. Q. Su, N. Matsusaka, S. Tsuda (1998). Detection in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs Mutation Research, 419(1-3,1-3), 13-20 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | DNA damage (comet assay) in multiple organs of mice exposed orally | | | | | |
| HERO ID: | 5447470 | | | | | |
| Domain | Metric | Rating [‡] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Test Animal Characteristics | Medium | × 2 | 4 | Male CD-1 mice, 7 wks old, were obtained from Charles River, Japan. Minor details (initial body weight and health status) are missing, but unlikely to have a substantial impact on results. | |
| | Metric 14: Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Husbandry conditions (temperature, RH, light/dark cycle, and diet) were reported and adequate. Cage conditions were not reported but unlikely to substantially impact this short duration study | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | N=4; this number is slightly smaller than typical for this study type/outcome | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Assessment methodology is partially described and cited to other publications. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | Outcome assessment execution is briefly reported. Controls were untreated; thus, the appropriate timing of outcome assessment is uncertain. | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling was adequate (50 nuclei/organ/animal). | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not applicable. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Negative control data are presented in Table 1 and appear consistent across experiments. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight and food/water intake of study groups are not reported. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | There were no deaths, morbidity, or clinical signs. No information on outcomes unrelated to exposure was reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | High | × 1 | 1 | Statistical methods (1-way ANOVA and Dunnett test, p<0.05) are reported and appropriate | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for all groups, time points, and target organs are reported in Table 1 (mean±SEM). | |
| Overall Quality Determination [‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |

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

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Study Citation: | B. E. Butterworth, T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Jirtle, G. Michalopoulos, S. Strom (1989). Use of primary cultures of human hepatocytes in toxicology studies <i>Cancer Research</i> , 49(5,5), 1075-1084 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | UDS on primary hepatocytes - CCl4 and CHCl3 | | | | | |
| HERO ID: | 6265 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were clearly identified as carbon tetrachloride and chloroform. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substances were purchased from Fischer. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Test substance was reported as ACS certified. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative controls were included (media and DMSO controls). | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Although the study did not identify positive controls, many chemicals were included, several of which were strong genotoxic carcinogens. Two test substances (2-acetylaminofluorene and dimethylnitrosamine) are commonly used as positive control substances for a variety of genotoxicity assays and yielded positive responses in the present article. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were briefly described and cited elsewhere (Butterworth, et al., 1983, Butterworth, et al., 1987), but appeared appropriate for the outcome of interest. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | It was reported that test substances were prepared "in DMSO or water" but it was not explicitly specified which test substances were dissolved in which. However, it was noted that the choice depended on solubility, so this is not considered to have substantially impacted results. Test substance storage was not reported, but the is appropriate given the study design (single-dose administration). | |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Details of exposure administration are not reported; however, they are unlikely to have a substantial impact on results. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure doses were reported without ambiguity in Tables 2 and 3. | |
| Continued on next page ... | | | | | | |

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

Data Type: UDS on primary hepatocytes - CCl4 and CHCl3

HERO ID: 6265

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

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| Study Citation: | B. E. Butterworth, T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Jirtle, G. Michalopoulos, S. Strom (1989). Use of primary cultures of human hepatocytes in toxicology studies <i>Cancer Research</i> , 49(5,5), 1075-1084 | | | | |
|--|---|---------------------|------|-------|--|
| Data Type: | UDS on primary hepatocytes - CCl4 and CHCl3 | | | | |
| HERO ID: | 6265 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Automated measurements were made. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | The use of primary human cells introduces confounding variables (differences in age, sex and health of patients were noted). This, however, is unlikely to have a substantial impact on results, as these confounding variables were unavoidable for primary human samples and were controlled for appropriately; 1) there were several donors in the study, 2) details differentiating donor age/sex/health status and hepatocyte isolation were described, and 3) the data from multiple donors was not pooled or directly compared. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on outcome differences unrelated to exposure were not reported for each study replicate or group. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical methods were reported and appropriate. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Scoring and evaluation criteria were reported and appropriate. |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Cytotoxic endpoints (description of pyknotic cells) were defined and appropriate. |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | The authors report that Table 3 contains typical results selected from several experiments. Data pertaining to CCL4 in rat hepatocytes is not included. It is unclear if the data were omitted or the assay was not run. |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.3 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 108: **In vitro** evaluation results of Garberg et al., 1988 for genotoxicity (alkaline elution) assay

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|--|
| Study Citation: Garberg, P; Akerblom, EL; Bolcsfoldi, G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution Mutation Research, 203(3), 155-176 | | | | | |
| Data Type: Genotoxicity (Alkaline elution) assay | | | | | |
| HERO ID: 7271 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The source of the test substance reported including manufacturer, but the batch/lot number not provided. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Although the authors did not report the purity of the chemical, it may be of a minor concern since the chemical is from a standard company. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Test authors report using a concurrent negative control group. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | The authors do not report a concurrent positive control group. But it may not be a concern since they have used known genotoxic chemicals in this study. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were reported in detail. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | The QC part of this test criteria may not be applicable. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Not Rated | NA | NA | This may not be applicable since the test chemical was purchased from a commercial vendor and can be used with or without storage. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration were reported. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses/concentrations were reported. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Number of exposure groups and concentration spacing were reported. |
| Metric 13: | Metabolic Activation | High | × 1 | 1 | Tests were done with and without metabolic activation. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The authors used a standard genotoxicity test model. |
| Metric 15: | Number per Group | High | × 1 | 1 | The authors reported the number of cells per group. |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was reported. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was consistent. |
| Metric 18: | Sampling Adequacy | High | × 2 | 2 | Adequate. |

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| Study Citation: | Garberg, P; Akerblom, EL; Bolcsfoldi, G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution Mutation Research, 203(3), 155-176 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Genotoxicity (Alkaline elution) assay | | | | | |
| HERO ID: | 7271 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | The authors did not conduct statistical analysis. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Data interpretation was consistent. | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Authors reported cytotoxicity data (cell viability). | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data was reported for all doses. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.2 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 109: **In vitro** evaluation results of Sina 1983 for mutagenesis in rat hepatocyte assay

| Study Citation: | J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | DNA damage (SSB) in rat hepatocytes for CCl4 | | | | | |
| HERO ID: | 7323 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride with the correct CASRN. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was identified. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity of the test substance was not identified. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Negative controls were included. It was not specified whether the negative controls were treated with water, DMSO, or left untreated. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Dimethylnitrosamine was utilized as a positive control in each assay. Positive results were obtained from positive control groups. This compound requires metabolic activation and was also utilized as a validation of hepatocyte metabolism. | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were well-described. | |
| Metric 7: | Standards for Tests | High | × 1 | 1 | The QC criteria were adequate to demonstrate validity, acceptability, and reliability of this test. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The preparation of the test substance was reported. The storage of the test substance was not reported (single dose administration). | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were reported without ambiguity. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration (3 hr) was reported and appropriate for the outcome of interest. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Number of exposure groups and dose spacing was reported and appropriate. | |
| Metric 13: | Metabolic Activation | High | × 1 | 1 | This assay did not include an exogenous metabolic activation step, as the cells used were primary rat hepatocytes. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | Medium | × 2 | 4 | The identity and origin of the test model were reported. No additional information was provided. | |
| Metric 15: | Number per Group | Low | × 1 | 3 | The number of plates independently treated with CCl4 is not specified (although 2 replicates/plate was indicated). This may suggest the use of a single culture per concentration. | |

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| Study Citation: | J. F. Sina, C. L. Bean, G. R. Dysart, V. I. Taylor, M. O. Bradley (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Mutation Research: Environmental Mutagenesis and Related Subjects, 113(5,5), 357-391 | | | | |
|--|---|---------------------|------|-------|--|
| Data Type: | DNA damage (SSB) in rat hepatocytes for CCl4 | | | | |
| HERO ID: | 7323 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 5: Outcome Assessment | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the intended outcome of interest. |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment methodology was consistent across treatment groups. |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the outcome. |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. |
| Domain 6: Confounding / Variable Control | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial conditions were not reported for each study replicate or group. |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on disproportionate outcomes unrelated to exposure were not reported. |
| Domain 7: Data Presentation and Analysis | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis was not conducted. A three-fold increase in DNA single-strand breaks over negative controls was considered to be a positive result. Raw data are available for statistical analysis. |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The evaluation criteria (DNA single-strand breaks) are consistent with current standards. |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | The cytotoxicity of all compounds tested was measured by either trypan blue dye exclusion or release of glutamate-oxaloacetate transaminase (GOT) from the cells. The methods were adequately described for each cytotoxicity assay, but it was unclear which assay was used for CCl4. |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data were reported adequately. |
| Overall Quality Determination [‡] | | High | | 1.5 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 110: **In vitro** evaluation results of McCann et al., 1975 for Ames assay

| Study Citation: | J. McCann, E. Choi, E. Yamasaki, B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals Proceedings of the National Academy of Sciences, 72(12,12), 5135-5139 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | Ames assay | | | | | |
| HERO ID: | 8422 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | The substance was identified by name as carbon tetrachloride. | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | Carbon tetrachloride was obtained from Mallinckrodt | |
| | Metric 3: Test Substance Purity | Low | × 1 | 3 | Author stated that the purest grades available were used for all chemicals, but specific purity was not stated. | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | Unacceptable | × 2 | 8 | A concurrent negative control group was either not included or not reported. Authors note the numbers of spontaneous revertant colonies (specific to each strain) that were subtracted from the numbers but these may be based on historical data. | |
| | Metric 5: Positive Controls | Not Rated | NA | NA | no positive control was used, but treatment-related positive responses were observed for other compounds | |
| | Metric 6: Assay Procedures | Not Rated | NA | NA | Methods were cited to other publications and not well described | |
| | Metric 7: Standards for Tests | Not Rated | NA | NA | Not applicable for this study type | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 8: Preparation and Storage of Test Substance | Not Rated | NA | NA | Exposure methods cited to other publications with no details provided | |
| | Metric 9: Consistency of Exposure Administration | Not Rated | NA | NA | Exposure methods cited to other publications with no details provided | |
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | The highest test substance concentration tested was 10000 ug (10 mg) as reported in the table and footnotes | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | Not Rated | NA | NA | Exposure methods cited to other publications with no details provided | |
| | Metric 12: Exposure Route and Method | Unacceptable | × 1 | 4 | The number of exposure groups and concentration spacing were not reported, and dose-response was reportedly used to evaluate response. | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Aroclor-induced rat liver S9 metabolic activation was used and is appropriate for the Ames assay, but the system was not described. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Low | × 2 | 6 | Test model reported along with limited descriptive information. <i>S. typhimurium</i> is standard model. Strains tested are reported inconsistently. Table indicates that two strains (TA100 and TA1535) were tested; however, footnotes report that nonmutagenic compounds were tested in at least 4 strains. Source of the test model was not reported. | |

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| Study Citation: | J. McCann, E. Choi, E. Yamasaki, B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals Proceedings of the National Academy of Sciences, 72(12,12), 5135-5139 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Ames assay | | | | | |
| HERO ID: | 8422 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Not Rated | NA | NA | Assay methods cited to another publication without additional details | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment methods cited to another publication without additional details | |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment methods cited to another publication without additional details | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable to study type | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not reported. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial batch/lot number of organisms or models used per group was not reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay. | |
| | Metric 23: Data Interpretation | Unacceptable | × 2 | 8 | Evaluation criteria were not well described. In text, negative result is reported as <0.01 revertants/nmol, but in one table footnote, this is reported as the criterion for weakly mutagenic. Another footnote indicates that compounds were called non-mutagenic if there was no dose-response. | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Cytotoxicity endpoints were not defined and methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results. Footnotes indicate that compounds were tested "up to the maximum allowable concentration if the compound was inhibitory"; this may indicate testing to the limit of toxicity but it is not clear. | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Data reported as revertants per nmol or per plate. It is not clear which of 2 tested strains yielded the results. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 3.0 | | |
| Extracted | | No | | | | |
| Continued on next page ... | | | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 111: **In vitro** evaluation results of Callen et al., 1980 for *S. cerevisiae* mutagenicity study

| Study Citation: | D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in <i>Saccharomyces cerevisiae</i> Mutation Research, 77(1,1), 55-63 | | | | | |
|-------------------------------------|--|---------------------|------|-------|--|--|
| Data Type: | <i>S. cerevisiae</i> mutagenicity for CCl4 | | | | | |
| HERO ID: | 10054 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Appropriate concurrent negative control groups were included. | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. The test substances used in the study exhibited dose-related increased frequencies of gene mutations (indicative of effective assay conditions). | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay methods and procedures were adequately described. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | Test substance preparation was reported; methods took into account the volatility of the test substance (i.e., the use of screw-capped centrifuge tubes). Test substance storage was not reported, but this omission is unlikely to substantially impact the study results (single-dose administration). | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate (based on observations of positive responses). Preliminary experiments were used as an aid to determine the appropriate exposure time. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The study used three exposure groups plus controls; substantial toxicity was observed at the highest tested dose. | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to this study design. The <i>Saccharomyces cerevisiae</i> cells used in the study contain cytochrome P-450, capable of converting chemicals to reactive products. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The identity, source, and relevant genetic details for the various strains of <i>S. cerevisiae</i> were reported and appropriate for the outcome of interest. | |
| Metric 15: | Number per Group | High | × 1 | 1 | At least 5 plates were used per treatment condition. | |
| Domain 5: Outcome Assessment | | | | | | |

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| Study Citation: | D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in <i>Saccharomyces cerevisiae</i> Mutation Research, 77(1,1), 55-63 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | S. cerevisiae mutagenicity for CCl4 | | | | | |
| HERO ID: | 10054 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology is appropriate for the outcome of interest. The methods used permitted the detection of gene revertants, gene conversion, and mitotic recombinants. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this endpoint. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to this study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | No differences among treatment group parameters were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on outcome differences unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analyses are not required by study type (data for individual plates were pooled, so that independent statistical analyses are not possible). Data were presented as the number of revertants, recombinants, or convertants per 10 ⁵ survivors (pooled data); data for numbers of revertants, recombinants, or convertants per plate (and including a measure of variation) were not reported. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The criteria for a positive result was explicitly specified (i.e., at least a doubling of colonies compared to the controls). | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | A measure of cytotoxicity (percent survival compared to control, measured by total number of colonies counted) was determined concurrently with the mutagenicity assay results. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data were reported by exposure group. | |
| Overall Quality Determination [‡] | | High | | 1.2 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 112: **In vitro** evaluation results of Uehleke et al., 1977 for mutagenicity assay in *S. typhimurium*

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity <i>Xenobiotica</i> , 7(7,7), 393-400 | | | | | |
| Data Type: mutagenicity assay in <i>S. typhimurium</i> | | | | | |
| HERO ID: 10071 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified as CCl4 and CHCl3. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not reported. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity and/or grade of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Study authors did report using a negative control (spontaneous mutation), but details regarding the negative control group were not reported |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A concurrent positive control was not used but may not be required for this study. The response of some known carcinogens tested in the study were positive. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were partially described, but appeared to be appropriate |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported (dissolved in 5 ul of ethanol). The test substance storage was not reported, but this is appropriate given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated group |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration was reported in the results without ambiguity (8 mM). Doses for bacterial reverse mutation assays are usually reported in terms of ug/plate, but the volume of the incubation mixture (1.5 mL) for one plate is given, so ug/plate could be determined. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Medium | × 2 | 4 | The exposure duration was reported and appropriate (60 minutes). Current standards indicate that 20 minutes is appropriate for the preincubation method, but this is not expected to have had a substantial impact on results. |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | Only one exposure concentration was tested. It was noted that the concentration used was determined in a preliminary experiment to not reduce survival of bacteria by more than 10%, but the range of doses found to produce cytotoxicity in this preliminary assay were not reported. Cytotoxicity was assessed concurrently with the present results, but results were not reported. It is not clear whether the dose utilized was high enough to detect a positive result. |

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| Study Citation: | H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity <i>Xenobiotica</i> , 7(7,7), 393-400 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | mutagenicity assay in <i>S. typhimurium</i> | | | | | |
| HERO ID: | 10071 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Low | × 1 | 3 | Assays were conducted with metabolic activation described only as metabolically active rabbit liver microsomes (5 mg). | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model was reported with limited descriptive information. The test model was routinely used for the outcome of interest. (<i>S. typhimurium</i> strains TA1535 and TA 1538). The source of the bacteria strains was not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | 4 replicates per experimental condition were utilized. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | Details on outcome assessment protocol execution were limited to determine if it was carried out consistently across the treated and control group. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no confounding variables noted in the study | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding variable unrelated to exposure were identified | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistics were not used to assess increased revertants/plate from the control. It was reported that the results was the mean of 4 different incubation mixtures, but the results for the different incubation mixtures was not reported. Standard deviations to the mean was not provided; however, the number of mutations was reported < 10. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable. | |
| | Metric 23: Data Interpretation | Unacceptable | × 2 | 8 | Mutagenicity was expressed as cfu counted on the plates for his+ revertants per cfu of his- survivors; however, the evaluation criteria was not reported. The reporting of this ratio is inconsistent with current standards (absolute number of revertants per plate). Raw data is not reported to determine absolute number of revertants per plate. Therefore, this method of data interpretation and reporting is considered unacceptable. | |
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Study Citation: H. Uehleke, T. Werner, H. Greim, M. Kramer (1977). Metabolic activation of haloalkanes and tests in vitro for mutagenicity *Xenobiotica*, 7(7,7), 393-400
 Data Type: mutagenicity assay in *S. typhimurium*
 HERO ID: 10071

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 113: **In vitro** evaluation results of De Flora 1981 for mutagenicity assay in *S. typhimurium*

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: S. De Flora (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis, 2(4,4), 283-298 | | | | | |
| Data Type: mutagenicity assay in <i>S. typhimurium</i> | | | | | |
| HERO ID: 14322 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified as carbon tetrachloride (CCl4) and formaldehyde (HCHO). |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substances were reported (CCl4: Merck; HCHO: BDH). Lot/batch numbers were not reported, but these test substances are not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity and/or grade of the test substance was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Study authors did report using a negative control (spontaneous mutations) for each strain, but it was unclear what the identity of the negative controls were (untreated or solvent). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | A concurrent positive control was not specified but may not be required for this study. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were partially described, but appeared to be appropriate. The methods section refer to original technical descriptions of the assays (Ames et al., 1975) |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance preparation was inadequately reported ("dissolved and/or diluted either in twice distilled water or in [DMSO]"). This is not likely to have affected study results. Test substance storage was not reported, but this is appropriate given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated group |
| Metric 10: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | The upper test concentration limit was reported in the results with the limit dependent on Toxicity and based on preliminary data. However, the other doses tested for these compounds were not reported. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Not Rated | NA | NA | The exposure duration was not reported; however, it was noted that the assay were performed according to the original technical description (Ames et al., 1975) |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | Only one concentration (upper limit) was reported in the results. It was noted that solutions were diluted to a narrow range of concentrations below solubility or toxicity levels (based on preliminary data); only the upper limit was reported and the other tested concentrations were not reported. Therefore, the number of exposure groups and dose spacing were not reported. |
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| Study Citation: | S. De Flora (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis, 2(4,4), 283-298 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | mutagenicity assay in <i>S. typhimurium</i> | | | | | |
| HERO ID: | 14322 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (Aroclor-pretreated rats liver S9 fractions). Some details regarding the metabolic activation system were not fully described. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model was reported with limited descriptive information. The test model was routinely used for the outcome of interest. (<i>S. typhimurium</i> strains TA1535, TA1537, TA1538, TA98, and TA100). The source of the bacteria strains was not reported. | |
| | Metric 15: Number per Group | High | × 1 | 1 | It was noted that each concentration was tested in duplicate or triplicate. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across the controls and treated groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no confounding variables noted in the study | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding variable unrelated to exposure were identified | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistics were not used to assess increased revertants/plate from the control. The number of net revertants at the top level of the dose-response curve and the mutagenic potency was calculated (revertants/nmol compound). Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria were reported; a key for the interpretation of the results is included in the paper. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | Survival rats of the test strains were determined. Preliminary assays were conducted in or to determine toxicity to inform assay doses tested. The highest dose tested for CCl ₄ was based on its cytotoxicity level. Results for cytotoxicity were not reported. | |
| Continued on next page ... | | | | | | |

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

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 114: **In vitro** evaluation results of De Flora et al., 1984 for bacterial reverse mutation (direct plate incorporation) *S. typhimurium*

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test <i>Mutation Research</i> , 133(3,3), 161-198 | | | | | |
| Data Type: Bacterial reverse mutation (direct plate incorporation) <i>S. typhimurium</i> | | | | | |
| HERO ID: 17980 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified as carbon tetrachloride and formaldehyde. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Ames reversion test: Study authors acknowledged using a concurrent negative control group, but it is unclear whether negative control was untreated or a vehicle solvent. Methods for this assay were partially cited to other publications that may contain this information. This is not expected to have substantially impacted results. |
| Metric 5: | Positive Controls | High | × 2 | 2 | Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-N-oxide, cyclophosphamide, and sodium azide) were included and yielded appropriate positive results. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were briefly described; however, it was noted that the plate-incorporation test was conducted according to procedures described in Ames et al., 1975; the reported details suggest the assay was appropriate. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, this is appropriate given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated groups; applications were controlled for evaporation. |
| Continued on next page ... | | | | | |

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| Study Citation: | S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Bacterial reverse mutation (direct plate incorporation) <i>S. typhimurium</i> | | | | | |
| HERO ID: | 17980 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | Concentrations tested were not reported; however, it is noted that various dilutions were performed by a geometric ratio of 2, starting with its solubility or toxicity limit. The highest dose tested can also be estimated based on the reported mutagenic potency value (potency < 0.002 for CCl ₄ and < 0.08 for HCHO) was calculated by dividing the arbitrary value of 100 revertants by the nmoles corresponding to the maximum dose tested). | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | Not Rated | NA | NA | The exposure duration was not reported; however, it was noted that the assay was performed according to the original technical description (Ames et al., 1975) | |
| | Metric 12: Exposure Route and Method | Low | × 1 | 3 | The number of dose groups was not reported; however, it was noted that the compound was tested at various dilutions performed by a geometric ratio of 2 starting from its solubility or toxicity limit. | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details, but not fully described; it was noted that the study was conducted according to Ames et al., 1975 where they describe the preparation of the S9 mix | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model was reported and is routinely used for the outcome of interest (<i>S. typhimurium</i> strains TA1535, TA1537, TA1538, TA98, and TA100, TA97); sourced from Ames Lab (Department of Biochemistry, University of California, Berkeley, CA). | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | It was noted that each concentration was tested in duplicate or triplicate. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across groups | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |

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

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|---|
| Study Citation: S. De Flora, P. Znacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test <i>Mutation Research</i> , 133(3,3), 161-198 | | | | | |
| Data Type: Bacterial reverse mutation (preincubation) in <i>E. coli</i> | | | | | |
| HERO ID: 17980 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified as carbon tetrachloride |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported (Merck). The test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride. This is not expected to have substantially impacted results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The use of a solvent control was reported |
| Metric 5: | Positive Controls | High | × 2 | 2 | Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, and cyclophosphamide) were included and yielded appropriate positive results. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were partially described; the reported details suggest the assay was appropriate. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, this is appropriate given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated groups; applications were controlled for evaporation. |
| Metric 10: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | Concentrations tested were not reported. Qualitative results were not associated with specific test concentrations. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate for the study type |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | The number of exposure groups and dose spacing was not reported |
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| Study Citation: | S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation (preincubation) in E. coli | | | | | |
| HERO ID: | 17980 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details not fully described; it was noted that the S9 mix was prepared according to Ames et al., 1975. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy). | |
| | Metric 15: Number per Group | High | × 1 | 1 | It is noted that results were confirmed in at least 3 separate experiments. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoints of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across groups | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not conducted; independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria were reported | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | cytotoxicity was defined as survival; the assay evaluated and compared survival to a repair-deficient strain | |
| | Metric 25: Reporting of Data | | × 2 | NA | Data for exposure-related findings were presented qualitatively. Data for each study group was not reported. Qualitative results were not associated with specific test concentrations. Data were not reported for controls. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.8 | | |
| Extracted | | No | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 116: **In vitro** evaluation results of De Flora et al., 1984 for DNA-repair test (liquid micromethod) in *E. coli*

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|-------------------------------|---------------------|------|-------|---|
| Study Citation: S. De Flora, P. Znacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test <i>Mutation Research</i> , 133(3,3), 161-198 Data Type: DNA-repair test (liquid micromethod) in <i>E. coli</i> HERO ID: 17980 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified as carbon tetrachloride and formaldehyde. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Study authors acknowledged that compounds were dissolved in water, DMSO, or ether, but did not specify that negative concurrent vehicle controls were utilized. Figure 1 depicts a plate map for each compound and is accompanied by test describing the mixture (media, bacteria, S9, test substance, etc.) that was added to each well of the plate. None of the wells in the plate map were a negative control. The responses were compared between repair-deficient bacteria and repair-proficient strains, so it is not clear that a concurrent negative vehicle control is required and this is considered acceptable. However, a concurrent vehicle control would help to ensure confidence in the reliability of results. |
| Metric 5: | Positive Controls | Medium | × 2 | 4 | Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as 9,10-dimethylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-N-oxide, cyclophosphamide, and sodium azide) were included and appeared to yield positive results, although the criteria for a positive or negative result were not clearly defined. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were partially described with some omissions; however, it was noted that the procedure was similarly conducted as described by Kada et al., (1980) for the rec-assay; the reported details suggest the assay was appropriate. The study was designed to calibrate the method and support the use of this assay with the aid of other DNA-repair tests. |
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| Study Citation: | S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA-repair test (liquid micromethod) in E. coli | | | | | |
| HERO ID: | 17980 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 7: Standards for Tests | Not Rated | NA | NA | Not applicable for this study | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 8: Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, this is appropriate given the study design (single-dose administration). | |
| | Metric 9: Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated groups; applications were controlled for evaporation. | |
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | Not all concentrations tested were reported; however, minimal inhibitory concentrations were reported in the results. | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate for the study type | |
| | Metric 12: Exposure Route and Method | Low | × 1 | 3 | The number of dose groups was not reported; however, It was noted that the initial concentration of each compound was governed by its solubility or toxicity based on preliminary assays. The compounds were further diluted for a total of eight 2-fold dilutions. | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Assays were conducted with and without metabolic activation (10% liver S9 fractions from Aroclor-treated Sprague-Dawley rats). The method of preparation was reported with some details not fully described; it was noted that the S9 mix was prepared according to Ames et al., 1975. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy). | |
| | Metric 15: Number per Group | High | × 1 | 1 | It was noted that each compound was assayed in at least 5 separate experiments to check reproducibility. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoint of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across groups | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
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| Study Citation: | S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
|--|--|------------------------------|------------------|-------|--|--|
| Data Type: | DNA-repair test (liquid micromethod) in E. coli | | | | | |
| HERO ID: | 17980 | | | | | |
| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not conducted; independent statistical analysis is not possible. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | Evaluation criteria were reported, but it was unclear what constituted a positive result. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | cytotoxicity data was not presented. It was noted that the doses tested were based on solubility or toxicity for bacteria inferred from preliminary assays | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Data for the outcome was presented for each strain, but not for every dose tested. It is not clear if the values reported represent all replicate experiments. Data were not presented for controls. | |
| Overall Quality Determination [‡] | | Medium → Medium [§] | | 1.8 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: S. De Flora, P. Zanicchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
| Data Type: Spot test in <i>E. coli</i> | | | | | |
| HERO ID: 17980 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substances were identified as carbon tetrachloride and formaldehyde. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The sources of the test substances were reported (CCl4: Merck; HCHO: BDH). The test substances are not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity and/or grade of the test substance was not reported; it was noted that most of the substances tested were reagent grade pure compounds, but was not specific to carbon tetrachloride or formaldehyde. This is not expected to have substantially impacted results. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Testing of a negative untreated or solvent control for the spot test was not reported. The responses were compared between repair-deficient bacteria and repair-proficient strains, so it is not clear that a concurrent negative vehicle control is required and this is considered acceptable. However, a concurrent vehicle control would help to ensure confidence in the reliability of results. |
| Metric 5: | Positive Controls | High | × 2 | 2 | Test substances were not specified as positive controls, but several compounds routinely used as positive controls in the bacterial reverse mutation assay (such as benzo[a]pyrene, N-methyl-N'-nitro-N-nitrosoguanidine, and sodium azide) were included and yielded appropriate positive results. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were partially described; the reported details suggest the assay was appropriate. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The test substance preparation was reported (dissolved in DMSO). Storage of the test substance was not reported; however, this is appropriate given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were reported to be administered consistently across the control and treated groups; applications were controlled for evaporation. |
| Metric 10: | Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | Concentrations tested were not reported. Qualitative results were not associated with specific test concentrations. |
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| Study Citation: | S. De Flora, P. Zanacchi, A. Camoirano, C. Bennicelli, G. S. Badolati (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutation Research, 133(3,3), 161-198 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Spot test in E. coli | | | | | |
| HERO ID: | 17980 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported and appropriate for the study type | |
| | Metric 12: Exposure Route and Method | Unacceptable | × 1 | 4 | The number of exposure groups and dose spacing was not reported | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | The spot assay was conducted only in the absence of metabolic activation. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model was reported and is used for the outcome of interest (E.coli WP2, WP67, CM871); source from Monti-Bragadin lab (Institute of Microbiology, University of Trieste, Italy). | |
| | Metric 15: Number per Group | Unacceptable | × 1 | 4 | The assay was repeated only if no inhibition was detected. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodologies were appropriate for the endpoint of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across groups | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This method is not applicable to the outcome. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was not conducted; independent statistical analysis is not possible. However, statistical analysis is not necessarily required for this assay. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria were reported | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | cytotoxicity endpoints were not defined and it cannot be determined if cytotoxicity was accounted for in the interpretation of the study results. | |
| | Metric 25: Reporting of Data | Unacceptable | × 2 | 8 | Data for exposure-related findings were presented qualitatively. Data for each study group was not reported. Qualitative results were not associated with specific test concentrations. Data were not presented for controls. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.1 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 118: **In vitro** evaluation results of Zeiger et al., 1988 for Salmonella mutagenicity assay

| Study Citation: | E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans (1988). Salmonella mutagenicity tests: IV: Results from the testing of 300 chemicals Environmental and Molecular Mutagenesis, 11(Suppl 12,Suppl 12), 1-158 | | | | | |
|-------------------------------------|---|---------------------|------|-------|---|--|
| Data Type: | Salmonella mutagenicity assay for CCl4 | | | | | |
| HERO ID: | 24516 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance was clearly identified as carbon tetrachloride. A CASRN was also provided (56-23-5). | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source of test substance was identified as a manufacturer (J.T. Baker Chemical). Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Negative controls were concurrently run. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Positive controls were run concurrently and elicited a positive response (at least a two-fold increase in revertants). | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay procedures were partially described partially cited to other publications. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable to this study type. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was prepared in DMSO. Storage was not reported, but was not expected to have an impact on the assay. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure was administered consistently across the study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity in Tables 58.1 and 58.2. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported and appropriate (20 minutes followed by 2 days incubation). | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Exposure groups and dose spacing was justified. Half-log dose intervals up to toxic dose (initially determined) were used in this study. | |
| Metric 13: | Metabolic Activation | Medium | × 1 | 2 | Study authors reported exposures were conducted in the presence of metabolic activation and the type and source and concentration in final culture were described. However, preparation was cited to another publication. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | Cell lines were obtained from Dr. Bruce Ames (University of California, Berkeley). These strains are routinely used for the outcome of interest. | |
| Metric 15: | Number per Group | High | × 1 | 1 | Each dose was tested in triplicate. | |
| Domain 5: Outcome Assessment | | | | | | |

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| Study Citation: | E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans (1988). Salmonella mutagenicity tests: IV: Results from the testing of 300 chemicals Environmental and Molecular Mutagenesis, 11(Suppl 12,Suppl 12), 1-158 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Salmonella mutagenicity assay for CCl4 | | | | | |
| HERO ID: | 24516 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome methodology was reported and sensitive for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment was carried out consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable for this study. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Outcomes were assessed by automated measurements. It is noted that the study indicated that chemicals were coded (laboratories were not aware of the identity of the chemical being tested). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | There were no confounding variables in test design and procedures. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables in outcomes unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Data are presented as means ±SEM (statistical analysis not required by study type). | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Scoring and evaluation criteria were reported and appropriate. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | An initial toxicity assay was performed to determine the dose range for the mutagenicity assay. Toxicity was defined as concentrations that decreased the number of his+colonies, caused a clearing of the density of the background lawn, or both. The method was cited to another publication. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data were shown by exposure group. | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 119: **In vitro** evaluation results of Simmon et al., 1977 for bacterial reverse mutation

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water <i>Developments in Toxicology and Environmental Science</i> , 2, 249-258 | | | | | |
| Data Type: Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: 29451 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by name (carbon tetrachloride). |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The source of the test substance was reported incompletely (reported as a commercial supplier). Since the test substance is not expected to vary in composition and the test substance was obtained from a commercial supplier, the omitted details are unlikely to have a substantial impact on the results. |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | Purity and grade of test substance were not reported ("reagents of the highest available purity"). It was indicated that purity was not determined for most chemicals. This is not expected to have impacted results, as the test substance was obtained from a commercial supplier. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | A concurrent negative control (solvent control) was used, but the control response was not described. |
| Metric 5: | Positive Controls | Low | × 2 | 6 | A concurrent positive control was used (unnamed), but the control response was not described. |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Methods and procedures were partially described and cited in another publication (Ames et al., 1975), but appeared to be appropriate for the assays in desiccators (bacteria) and in suspension (yeast); some details (e.g., cell density for the bacterial assay) were reported incompletely. Special test conditions were used to account for the volatility of the test substance. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to this study type. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The test substance preparation was reported (e.g., test chemical added to glass petri plate for bacterial assay); lack of storage conditions are not likely to substantially impact the study results given the study design (single-dose administration). |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | Details of exposure administration were reported or inferred from the text with few study details (or cited to Ames et al., 1975). Exposures were reportedly for "7 to 10 hours" (unclear if time varied among concentrations or different chemicals tested); however, these differences were not expected to substantially affect the study results. |
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| Study Citation: | V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: | 29451 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 10: Reporting of Doses/Concentrations | Unacceptable | × 2 | 8 | The amounts of test substance used were not reported. It was stated that a "wide range of doses was tested up to 5 mg/plate or a dose which gave a toxic response, whichever was lower." | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | Low | × 2 | 6 | Exposure duration was reported (7-10 hours for the bacterial assay, 4 hours for the yeast assay). The duration of these assays was presumed appropriate for the study type (given that positive responses were observed for the test chemical or other chemicals used in the study). It is possible that the exposure duration for the bacterial assay (7 to 10 hours) varied across dose levels. It is also possible that the variation in exposure duration was across test substances instead, so this metric is still considered acceptable. | |
| | Metric 12: Exposure Route and Method | Unacceptable | × 1 | 4 | The number of exposure groups and dose/concentration spacing were not reported. The study states (for all chemicals) that a wide range of doses were tested up to a given concentration or until a toxic concentration was achieved (whichever was lower). | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | The presence of a commonly used metabolic activation system (e.g., rat or mice liver cells cited to Ames et al., 1975) was re-ported in the study; however, some details were not described. These omissions are unlikely to have a substantial impact on the results. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. Reporting limitations are unlikely to have a substantial impact on results. | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The number of replicates per study group were not reported (though procedures were reportedly consistent with Ames et al., 1975). Because there are no error bars in any graphs, it is considered likely that only one plate per dose was included in the study design, which is lacking. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Low | × 1 | 3 | It is not clear that the exposure duration (7-10 hr) and post-exposure incubation time ("approximately" 40 hrs) were equal for all doses of a test substance. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to this study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |

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| Study Citation: | V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water <i>Developments in Toxicology and Environmental Science</i> , 2, 249-258 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Bacterial reverse mutation for CCl4 | | | | | |
| HERO ID: | 29451 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | There were no reported differences among study group parameters (e.g., test substance, cells used) identified that could influence the outcome assessment. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variable unrelated to exposure were reported or identified. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Low | × 1 | 3 | Statistical analysis was presumably not conducted, and insufficient information was provided for independent statistical analysis (data not reported quantitatively or graphically for CCl4 due to negative response, as determined by study authors). However, statistical analysis is not necessarily required for the bacterial reverse mutation assay. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | Scoring and/or evaluation criteria were not reported and the omissions are likely to have a substantial impact on interpretation of the results. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | Cytotoxicity endpoints were evaluated, but the methods of measurements were not fully described or reported. | |
| | Metric 25: Reporting of Data | | × 2 | NA | Data presentation was inadequate (no data was shown). | |
| Overall Quality Determination [‡] | | Unacceptable** | | 2.6 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 120: **In vitro** evaluation results of **Brams et al., 1987** for **Ames** and **SOS Chromotest**

| Study Citation: | A. Brams, J. P. Buchet, M. C. Crutzen-Fayt, C. De Meester, R. Lauwerys, A. Leonard (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure) <i>Toxicology Letters</i> , 38(1-2,1-2), 123-133 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Ames and SOS Chromotest for CCl4 | | | | | |
| HERO ID: | 51352 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride (CCl4). | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Test substance was purchased from a manufacturer (Merck). A lot number was also provided. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | The purity/grade of the test substance was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study authors reported using negative (vehicle-only) controls. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | The study reported using reference mutagens for the Ames assay (2-NF and NaN3 as direct mutagens and 2-AA as an indirect mutagen). For the SOS chromotest, 4-nitroquinoline oxide was used as a direct mutagen. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | For the Ames assay, most of the methods/procedures are described, but another publication is also cited (Maron and Ames 1983). For the SOS chromotest, methods were partially described and also cited to the procedure recommended by the manufacturer of the kit (Organics). | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for this study. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | The study indicated that only freshly prepared solutions were tested. The lack of information on storage is not likely to impact the study results. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures appeared to be administered consistently across study groups (both assays). | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | A range of doses was provided. For the Ames assay, 3 concentrations from 100 to 1000 ug/plate were tested; for the SOS chromotest, 7 dilutions from 15.4 ng/mL to 1.54 mg/mL were tested. At least two concentrations were reported without ambiguity for each assay. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported and appropriate for the study types (Ames test 48 hours; SOS chromotest 2 hours). | |
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Study Citation: A. Brams, J. P. Buchet, M. C. Crutzen-Fayt, C. De Meester, R. Lauwerys, A. Leonard (1987). A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure) Toxicology Letters, 38(1-2,1-2), 123-133
 Data Type: Ames and SOS Chromotest for CCl4
 HERO ID: 51352

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---------------------|---------------------|------|-------|--|
| Metric 22: | Data Analysis | Not Rated | NA | NA | There is no indication that statistical analyses were performed. The study indicates that for the Ames assay, data were evaluated as the mean +/- SD of 3 plates (not shown). For the SOS chromotest, quantitative measurements were obtained using a photometer. |
| Metric 23: | Data Interpretation | Medium | × 2 | 4 | For the Ames assay, the evaluation criteria were specified (at least a 2-fold increase in revertants in one strain, a dose-response, and a reproducible effect), For the SOS chromotest, the evaluation criteria are not clearly specified (based on quantitative photometer measurements and based on manufacturer's recommendations). |
| Metric 24: | Cytotoxicity Data | Medium | × 1 | 2 | For the Ames study, it was indicated that the background lawn of the plates was examined to detect toxic effects; the study results indicate that viable counts in the bacterial cultures were very similar (no additional information provided). For the SOS chromotest, it was indicated that a viability control was performed at each dilution of the test substance; no toxicity was reported for CCl4. |
| Metric 25: | Reporting of Data | Medium | × 2 | 4 | Negative data are reported qualitatively. No quantitative data for CCl4 are provided in the study report. |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 121: **In vitro** evaluation results of Nakamura et al., 1987 for DNA repair

| Study Citation: | S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA repair for CCl4 | | | | | |
| HERO ID: | 51515 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The source of the test substance was reported (Katayama chemical). Although a batch/lot number was not provided, the test substance is not expected to vary in composition. | |
| Metric 3: | Test Substance Purity | Medium | × 1 | 2 | The purity/grade of the test substance was not reported. However, it was indicated that chemicals were of the highest quality commercially available. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | Negative controls were reported; however, it is not clear if they were run concurrently with test substance (e.g., DMSO was one of the 151 chemicals tested in the assay). | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | Positive responses were observed for several of the 151 chemicals tested in this study (demonstrating that the test is capable of detecting a positive response) it is unclear if these were run concurrently with test substance. It is noted the list of chemicals tested included test substances used as positive controls in the Ames assay. | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were briefly described and partially cited to another publication (Oda et al., 1985). | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Based on the text and the properties of the test substance, it can be inferred that the test substance was prepared in DMSO (but this was not explicitly stated). Storage conditions were not reported. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures were administered consistently across study groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Since responses were negative for all doses tested, only the highest dose was reported (5300 ug/mL). | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration was reported (2 hours) and appropriate for the outcome of interest. | |
| Metric 12: | Exposure Route and Method | Unacceptable | × 1 | 4 | The number of exposure groups and/or spacing was not reported. Only the highest tested dose was reported (no rationale provided). | |
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| Study Citation: | S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | DNA repair for CCl4 | | | | | |
| HERO ID: | 51515 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Medium | × 1 | 2 | Method of preparing liver S9 fraction are only partially reported (i.e., prepared from rats pretreated with phenobarbital and 5,6-benzoflavone). | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Low | × 2 | 6 | The cell line was developed in house; limited details were provided. The system was described as novel (not yet routinely used to assess this outcome). | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The number of replicates per group was not indicated. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Medium | × 2 | 4 | Methods for outcome assessment were largely cited to another publication (Miller, 1972). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment was carried out consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding was not necessary for this study. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variable were reported in test design/procedure. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Data are presented qualitatively (i.e., reported as negative). Statistical analyses do not appear to have been performed (despite the use of the term 'significant' in the results section), but are not required by study type (fold changes can be used to evaluate the response). | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | It was indicated that a 2-fold increase in beta-galactosidase activity above background levels was considered a positive effect. The study authors further classified chemicals used in the study as potent inducers (6-fold changes), intermediate inducers (3-fold changes), or weak inducers (2-fold changes). | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results. | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Only one data point is reported (highest tested concentration); however, since the results were negative, this is unlikely to have a substantial impact on results. | |
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Study Citation: S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
 Data Type: DNA repair for CCl4
 HERO ID: 51515

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------|---------------------|------|-------|------------------------|
| Overall Quality Determination [‡] | | Unacceptable** | | 1.9 | |
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 122: **In vitro** evaluation results of **Direnzo et al., 1982** for DNA adducts

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: A. B. Direnzo, A. J. Gandolfi, I. G. Sipes (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA Toxicology Letters, 11(AMST,AMST), 243-252 Data Type: DNA adducts for CCl4 HERO ID: 75145 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by name as [radiolabeled] carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The radiochemical purity of the test substance (>99%) was reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Not Rated | NA | NA | This metric is not required by study type, as the measurement of radiolabeled test compound is the outcome. However, heat-denatured microsomes were used as incubation blanks in this study. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | The methods and assay procedures were sufficiently described. It is noted that another publication was cited to the methods section as well (Sipes and Gandolfi 1980). |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation of the test substance was described (mixed with unlabeled test substance and diluted in ethanol). Storage was not reported, but is not expected to impact the study results. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures appeared to be administered consistently. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The test concentration was reported without ambiguity. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration (60 min) was reported and appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | A single concentration was tested, but was appropriate for the outcome of interest. |
| Metric 13: | Metabolic Activation | Medium | × 1 | 2 | The activation system used (hepatic microsomal protein obtained from phenobarbital-treated rats) was appropriate; details of isolation/preparation were cited to other publications. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The test model, calf thymus DNA, was appropriate for the outcome of interest. The source of calf thymus DNA was reported (a manufacturer). |
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| Study Citation: | A. B. Drenzo, A. J. Gandolfi, I. G. Sipes (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA Toxicology Letters, 11(AMST,AMST), 243-252 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA adducts for CCl4 | | | | | |
| HERO ID: | 75145 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The experiment with microsomal bioactivation was repeated six times. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate/sensitive for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome was assessed consistently. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variables in test design/procedures were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | The study indicated that protein contamination could influence results. The authors took steps to specifically remove protein contamination from the isolated DNA. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | The mean and standard deviation (and including n, the number of experiments performed) were reported and were appropriate for the study type. | |
| | Metric 23: Data Interpretation | Medium | × 2 | 4 | Evaluation criteria were not explicitly stated (other than detection of radiolabeled test substance bound to DNA), however, the study reported test substances that covalently bound to DNA at levels exceeding 0.3 nmol/mg DNA. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | This metric is not applicable to the study design, as no cells were utilized. | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data for the radiolabeled CCl4 group were reported adequately. Data for binding to untreated DNA was not shown. | |
| Overall Quality Determination [‡] | | High | | 1.4 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 123: **In vitro** evaluation results of Perocco and Prodi 1981 for scheduled and unscheduled DNA synthesis

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|--|
| Study Citation: P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218 | | | | | |
| Data Type: Scheduled and unscheduled DNA synthesis for CC14 | | | | | |
| HERO ID: 75278 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | Medium | × 1 | 2 | The donor source and commercial source of the various test substances tested in this study were identified, but it was unclear which test substance originated from which source. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity of the test substance was identified to be between 97-99%. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Concurrent negative controls were included (DMSO). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were described adequately. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | High | × 1 | 1 | The preparation of the test substance was reported. The storage of the test substance was not reported (single dose administration). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposure administration was consistent across treatment groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | The final concentrations of the test substance used in the experiments was reported without ambiguity (in uL/mL). |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration (4 hr) was reported and appropriate for the outcome of interest. |
| Metric 12: | Exposure Route and Method | Low | × 1 | 3 | The number of exposure groups was reported (3 treatment groups plus control). Results for two of the three treatment groups were obtained from a representative toxicity experiment; subsequent experiments used a single dose. The concentrations selected in the representative assay were not useful for evaluating a dose-response. The study indicates that the test substance induced toxicity at tested concentrations. |
| Metric 13: | Metabolic Activation | Medium | × 1 | 2 | Rat liver phenobarbital-induced S9 mix was utilized. More detailed methods regarding metabolic activation were cited to other references. |
| Domain 4: Test Model | | | | | |
| Metric 14: | Test Model | Low | × 2 | 6 | It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, no further information regarding gender, age, or other important demographics were included. |
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| Study Citation: | P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | Scheduled and unscheduled DNA synthesis for CCI4 | | | | | |
| HERO ID: | 75278 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | It was reported that six replicates were used per experimental condition. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the intended outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment methodology was consistent across treatment groups. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study design. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, it is unclear whether the 6 replicates for each experimental condition originated from 6 individual donors. It is also unclear whether different experimental conditions were tested on the same set of lymphocytes (e.g. Dose 1 tested on lymphocytes originated from donors A, B, and C; Dose 2 tested on lymphocytes originating from donors D, E, and F; etc). | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | No confounding variables were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Unacceptable | × 1 | 4 | Statistical analysis was not conducted and raw data were not provided, preventing an independent statistical analysis. | |
| | Metric 23: Data Interpretation | Low | × 2 | 6 | The criteria for a positive response was not explicitly specified. | |
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | Scheduled DNA synthesis (SDS) was used as a measure of toxicity. Methods used to determine SDS were reported; however, cytotoxicity endpoints were not well-defined (i.e., the response that constituted a toxic effect). | |
| | Metric 25: Reporting of Data | Low | × 2 | 6 | Data were reported by exposure group; however, data for experiments conducted with and without activation were not reported separately. | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.8 | | |
| Extracted | | No | | | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 124: **In vitro** evaluation results of Loveday et al., 1990 for chromosome aberrations and sister chromatid exchanges

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|---|---|---------------------|------|-------|--|
| Study Citation: K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303 | | | | | |
| Data Type: SCEs and CAs for CCL4 | | | | | |
| HERO ID: 106324 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was clearly identified as carbon tetrachloride. A CASRN was also provided. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial supplier of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | The purity of the test substance (>99%) was reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Both media and solvent controls were used with each assay. |
| Metric 5: | Positive Controls | High | × 2 | 2 | Appropriate positive controls both with activation (cyclophosphamide) and without activation (mitomycin C) were used. A low- and high-dose positive control was used for the SCE assay. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay procedures were well described for SCE and CA experiments. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | This metric is not applicable to the study design. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | General information regarding test substance preparation was included (e.g., dissolving in solvent and preparation of stock solutions), but storage conditions were not provided. |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Information regarding exposure administration was reported and it appears that exposures were administered consistently across study groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Some of the doses/concentrations (including the highest test dose eliciting a negative response) were reported without ambiguity. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | Medium | × 2 | 4 | The exposure duration for each assay was reported; however, the time of exposure varied based on activation status, assay, and cell cycle delay. |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | Dose selections were described in detail. The highest dose in the SCE assay was based on solubility or toxicity (or 5 mg/mL if there were no issues). Doses the CA assay were based on cell cycle delay and toxicity noted in SCE assay. The study indicates that, for the SCE assay, dilutions were made to achieve 10 test concentrations in a half-log series covering a range of five logs; and at least 5 concentrations were spaced using two merged half-log scales for the CA assay. |
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| Study Citation: | K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | SCEs and CAs for CCL4 | | | | | |
| HERO ID: | 106324 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | The study authors reported exposures were conducted in the presence of metabolic activation and the type and source rat liver microsomal fraction) and volume in final culture were reported. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (CHO cells) was described in detail and were considered appropriate for the endpoints assessed. These cells are routinely used in assays of this type. | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | The number of replicates was not clearly indicated. It was noted that tests were repeated to confirm positive results if there was one or more elevated point, or if toxicity was too great. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | For each assay, the outcome assessment methodology was described in detail and appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcome assessment protocol was consistent across study groups. | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | The data in the appendix indicate that 1000+ chromosomes per concentration were evaluated for SCEs; 200 cells were evaluated per concentration for CAs (fewer than recommended by study type). | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The samples were reported to be coded and assessed by a single person. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | There were no confounding variables in test design or procedures that were reported by study authors. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | It was indicated that cells were found to be free of mycoplasma for all experiments. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Medium | × 1 | 2 | Statistical analyses were described and appropriate for data described. Data analysis was partially cited to other publications. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The criteria for a positive/negative result were described. For SCEs, a trend test and a 20% increase over solvent was designated as positive. For CAs, a positive response was defined as p (adjusted) < 0.05 based on analyses of increases in CAs over solvent controls. | |
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Study Citation: K. S. Loveday, B. E. Anderson, M. A. Resnick, E. Zeiger, H. E. Holden (1990). Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals Environmental and Molecular Mutagenesis, 16(4,4), 272-303
 Data Type: SCEs and CAs for CCL4
 HERO ID: 106324

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|------------------------------|---------------------|------|-------|---|
| | Metric 24: Cytotoxicity Data | Medium | × 1 | 2 | The study indicated that toxicity was determined as the percent of confluence of the cell monolayer in treated flasks in comparison with control flasks. Although measurements of toxicity were not fully reported, the summary table indicates doses above which toxicity was observed (when applicable; i.e., at 1490 ug/mL in the SCE assay for CCl4 without activation). The study indicates that toxicity was taken into account for these assays (highest dose evaluated was the one that allowed sufficient cells for analysis). |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data are shown for some (but not all) dose groups (based on the number of dose groups that were reportedly used in each assay). |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 125: **In vitro** evaluation results of Schiestl et al., 1989 for intrachromosomal recombination

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|---|---------------------|------|-------|---|
| Study Citation: R. H. Schiestl, R. D. Gietz, R. D. Mehta, P. J. Hastings (1989). Carcinogens induce intrachromosomal recombination in yeast Carcinogenesis, 10(8,8), 1445-1455 | | | | | |
| Data Type: Intrachromosomal recombination for CCl4 | | | | | |
| HERO ID: 188190 | | | | | |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test material was clearly identified as carbon tetrachloride. |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity/grade was not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Medium | × 2 | 4 | The study specifically indicated that solvent controls were used, and that this group was treated the same way as the other concentrations. For a few chemicals used in the study (other than CCl4), the solvent is specified as acetone or DMSO; it is inferred that water was the solvent for other chemicals (including CCl4). |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the study type. However, several known mutagens (including radiation) and substances that elicited positive results in carcinogenicity and/or Ames assays were tested and gave positive results. It was not explicit that these conditions were intended to serve as positive controls for this assay. |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Assay methods/procedures were described in detail. The volume of the test concentrations added to incubation tubes was not stated. |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Use of a standard is not required for this test method. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | It could be inferred that the test substance was dissolved in solvent. It was indicated that tubes were sealed. However, the stability of the test substance in that solvent (presumably water) was not demonstrated. Storage was not reported (but is not likely to impact the study results). |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | Exposures appeared to be administered consistently across study groups. |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Doses were reported without ambiguity in Table II. |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported (17 hours) and appeared to be appropriate for the outcome of interest. |
| Continued on next page ... | | | | | |

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| Study Citation: | R. H. Schiestl, R. D. Gietz, R. D. Mehta, P. J. Hastings (1989). Carcinogens induce intrachromosomal recombination in yeast Carcinogenesis, 10(8,8), 1445-1455 | | | | | |
|--|--|---------------------|------|-------|---|--|
| Data Type: | Intrachromosomal recombination for CCl4 | | | | | |
| HERO ID: | 188190 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 12: Exposure Route and Method | Medium | × 1 | 2 | The number of exposure groups was reported (4 plus controls). A rationale for dose selection was not provided. Spacing may not have been appropriate based on toxicity and the absence of a clear dose-response. | |
| | Metric 13: Metabolic Activation | Not Rated | NA | NA | This metric is not applicable to the study type (all experiments were conducted in the absence of S9). | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Medium | × 2 | 4 | The test model (<i>Saccharomyces cerevisiae</i> diploid strain RS112) used was described (with respect to genotypic features). The methods used to construct this strain in the lab were reported (not from a commercial source). | |
| | Metric 15: Number per Group | High | × 1 | 1 | Experiments were performed in triplicate with two plates per test concentration. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Low | × 2 | 6 | The outcome assessment was adequately described. However, it is not clear that the assessment was sensitive to the outcome of interest because increased intrachromosomal recombination was seen only in the presence of substantial toxicity (89% to 99%). | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups. | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | The study indicated that data derived from less than 5 colonies were not included. Based on data presented in Table II, the colony yield (HIS+) ranged from 232 to 8 across the range of doses tested. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | This metric is not applicable to the study type. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | No confounding variables in test design or procedures were reported. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | No confounding variables unrelated to exposure were reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Statistical analysis was not performed (and not required); data were evaluated as fold-increase over controls. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | The criteria for a positive response were specified. A minimum increase of 2-fold over the spontaneous frequency in a dose-dependent manner was regarded as evidence of inducibility. | |
| | Metric 24: Cytotoxicity Data | Low | × 1 | 3 | Cell viability was included; the authors did not discuss the potential impact of cytotoxicity on the observed results. Other than cell survival (as a percent), the authors did not define the cytotoxicity endpoint. | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|------------------------------|---------------------|------|-------|---|
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data were reported by exposure group (without a measure of variance). The study indicated that the same qualitative results were obtained in other strains. |
| Overall Quality Determination [‡] | | Medium | | 1.8 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 126: **In vitro** evaluation results of **Beddowes et al., 2003** for **DNA SSB (Comet assay), M1dG and 8-oxodG adducts**

| Study Citation: | E. J. Beddowes, S. P. Fau, J. K. Chipman (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress <i>Toxicology</i> , 187(2-3,2-3), 101-115 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | DNA SSB (comet assay), M1dG and 8-oxodG adducts, | | | | | |
| HERO ID: | 194414 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The substance was identified by chemical name, no CASRN was provided. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | A commercial source (Sigma-Aldrich) for the test substance was reported. | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity was not reported. | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | An untreated negative control was included. CCL4 was administered as a vapor so untreated control is appropriate | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | No positive controls were used; however positive responses were observed | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | Adequate assay procedures were described and appropriate for the outcomes of interest. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable to study type | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Test substance was applied using gas-tight syringes and cells culture in sealed flasks to minimize volatilization. Test substance storage was not reported but unlikely to affect this short term study. | |
| Metric 9: | Consistency of Exposure Administration | Medium | × 1 | 2 | The study reports that the method of administration was intended to allow the test substance to be absorbed as a vapor in a uniform manner. Volumes applied across groups were not reported. Measures were consistently taken to minimize evaporation prior to administration. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Initial concentrations (0.25, 1, and 4 mM) were estimated using partition coefficients calculated for hepatocytes using the headspace-vial equilibrium technique. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration (2hrs) was clearly reported and was appropriate for the outcomes of interest | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number of nonzero exposure groups (3) was appropriate and adequate to evaluate a dose-response. Cytotoxicity was statistically significant at the highest dose (92% of control). | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | The study used primary hepatocytes, metabolic activation was not required. | |
| Domain 4: Test Model | | | | | | |
| Continued on next page ... | | | | | | |

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

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

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|-------------------|---------------------|------|-------|---|
| Metric 25: | Reporting of Data | High | × 2 | 2 | Data were adequately reported for all outcomes and exposures. |
| Overall Quality Determination [‡] | | High | | 1.4 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 127: **In vitro** evaluation results of Selden et al., 1994 unscheduled DNA synthesis assay in rat hepatocytes

| Study Citation: | J. R. Selden, F. Dolbeare, J. E. Miller, J. H. Clair, K. Mcgettigan, J. A. Dijohn, G. A. Dysart, J. G. Deluca (1994). Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes Mutation Research, 315(2,2), 147-167 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | UDS assay in rat hepatocytes | | | | | |
| HERO ID: | 194433 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Identified as carbon tetrachloride, CASRN provided | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | The commercial source (Aldrich) is provided | |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Test substance purity is not reported | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study included a solvent (DMSO) negative control. | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Two positive controls were reported (MMS and 2-AAF) | |
| Metric 6: | Assay Procedures | High | × 1 | 1 | UDS was evaluated using two methods (flow cytometric and autoradiographic). Assay procedures for each method were adequately described. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable to this study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Unacceptable | × 1 | 4 | Preparation and storage were not described, except that CCL4 was dissolved in DMSO. Cells were cultured and treated in petri dishes, and the study did not describe any steps taken to reduce/prevent volatilization from the dishes. | |
| Metric 9: | Consistency of Exposure Administration | Low | × 1 | 3 | Details of application (e.g., volumes added) were not provided. The study did not report any measures taken to inhibit/prevent volatilization from petri dishes. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | A concentration range (1-10 ⁻² mM) was reported, but individual test concentrations and the exact number of test groups is not reported. | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was reported to be 18-20hrs. | |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | The exact number of exposure groups was not specified, but the text indicates that typical doses varied by half-log increments and the dose range was given. The maximum concentration, selected based on range-finding studies, was cytotoxic, exceeded solubility limits, or exceeded 10 mM. | |
| Metric 13: | Metabolic Activation | Not Rated | NA | NA | Metabolic activation was not necessary because the study was performed in primary rat hepatocytes. | |
| Domain 4: Test Model | | | | | | |
| Metric 14: | Test Model | High | × 2 | 2 | The test model (primary hepatocytes from male Crl:CD(SD)BR rats aged 6-9 wks) was appropriate for the outcomes assessed. | |

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| Study Citation: | J. R. Selden, F. Dolbeare, J. E. Miller, J. H. Clair, K. Mcgettigan, J. A. Dijohn, G. A. Dysart, J. G. Deluca (1994). Validation of a flow cytometric in vitro DNA repair (UDS) assay in rat hepatocytes Mutation Research, 315(2,2), 147-167 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | UDS assay in rat hepatocytes | | | | | |
| HERO ID: | 194433 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Medium | × 1 | 2 | Single assays were done on CCL4. For FCM, the assay was reportedly run in triplicate. For ARG assays yielding negative results, the highest nontoxic dose was analyzed to confirm. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The purpose of the study was to evaluate and validate a novel DNA repair assay (FCM) by comparing it to a conventional autoradiography assay. The methodologies were adequately described and appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | Medium | × 1 | 2 | It is unclear if there were any differences in the timing of outcome assessment across study groups because exposure duration was reported as a range. No other potential differences in outcome assessment were identified. | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | For the standard ARG study, 30 cells/slide were counted from 2-3 slides per dose. This may be lower than the recommend 50 cells/culture using six cultures, unless otherwise justified. For FCM, 1,000 cells/replicate were collected which may be lower than the typical 2,000 cells/replicate for other flow cytometry applications, however the lower sample sizes are not expected to have a great impact on study results. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Blinding is not applicable to the flow cytometric analysis. Cells in the ARG assay were counted using an automated colony counter and therefore blinding is not necessary. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | High | × 2 | 2 | Initial conditions were reported and consistent across groups. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | High | × 1 | 1 | A number of confounding variables related to the methods were recognized and discussed specific to flow cytometry (autofluorescence, low yields, high debris), and to the ARG method (cell-to-cell variability within a culture and culture-to culture variability within a dose. These were considered in statistical analysis and in the interpretation of the data. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Appropriate statistical analysis was used | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Evaluation criteria were adequately reported. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | The study tested cytotoxicity, which was adequately defined (< 90% viability of concurrent control). | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Data were presented for lowest effective response (ARG) and maximal and lowest effective responses (FCM). | |
| Overall Quality Determination [‡] | | Unacceptable** | | 1.4 | | |

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

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|-----------|--------|---------------------|------|-------|------------------------|
| Extracted | | No | | | |

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

* MWF = Metric Weighting Factor.

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

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

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lceil \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rceil_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

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

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

Table 128: **In vitro** evaluation results of Simmon and Tardiff 1978 for Ames assay

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

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| Study Citation: | V. F. Simmon, R. G. Tardiff (1978). The mutagenic activity of halogenated compounds found in chlorinated drinking water 2 417-431 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | Ames Assay | | | | | |
| HERO ID: | 194442 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | Low | × 1 | 3 | The number of test strains (2) is less than current standards however, the experiment was repeated at least once and the number of replicates (2/strain) per experiment was acceptable. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | Not Rated | NA | NA | Outcome assessment methodology was not reported, but may have been described in the cited publication. | |
| | Metric 17: Consistency of Outcome Assessment | Not Rated | NA | NA | Outcome assessment methodology was not reported, but may have been described in the cited publication. | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable to the study design | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable to the study design | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Numbers of cells in initial inoculates were not reported and could significantly impact the results | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | Not Rated | NA | NA | Statistical analysis was not reported; however statistical analysis is not always necessary for Ames assay interpretation. | |
| | Metric 23: Data Interpretation | Not Rated | NA | NA | Scoring and evaluation criteria were not reported but may have been provided in the cited publication | |
| | Metric 24: Cytotoxicity Data | Unacceptable | × 1 | 4 | Cytotoxicity endpoints were not defined, methods were not described, and it does not appear that cytotoxicity was considered in the study interpretation. | |
| | Metric 25: Reporting of Data | Unacceptable | × 2 | 8 | Data presentation for CCL4 was inadequate. The only information provided was a statement indicating that CCL4 was not mutagenic | |
| Overall Quality Determination [‡] | | Unacceptable** | | 3.1 | | |
| Extracted | | No | | | | |

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

* MWF = Metric Weighting Factor.

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

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

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

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

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

Table 129: **In vitro** evaluation results of Sivikova et al., 2001 for CA assay in ovine lymphocytes

| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | CA assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as carbon tetrachloride, no CASRN reported. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | A commercial source (Microchem, Bratislava, Slovak Republic) was reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity 99.8% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study included a solvent (DMSO) control | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Concurrent and appropriate positive control (ethylmethane-sulphonate) was used | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided that are presumed to provide more methodological details. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could effect the study results. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL) | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure duration (48 hrs) was reported. It is presumed that this duration is acceptable for this test model system. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index). | |
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| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|---|--|
| Data Type: | CA assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Low | × 1 | 3 | Metabolic activation was not included for this endpoint and no positive responses were reported. Metabolic activation was included in other assays reported in the same study. In is unclear why metabolic activation was not included for CAs. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Low | × 2 | 6 | The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old) | |
| | Metric 15: Number per Group | High | × 1 | 1 | Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the outcomes of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups | |
| | Metric 18: Sampling Adequacy | Low | × 2 | 6 | Text indicates that 100 well-spread metaphases were analyzed for CAs; however, Table 1 reports that 200 metaphases were determined per concentration. Guidance recommends 300 per concentration to conclude that a test chemical is clearly negative. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Use of coded slides or blinded evaluations were not specified. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial number of cells used per group was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis (<U+03C7>2-test) was appropriate for the outcome of interest and adequately described. Data were reported as Means with SD | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Mitotic index was evaluated and reported for each exposure concentration. High concentration was selected to achieve at least 50% reduction in mitotic index. For CCL4, high concentration MI was 1.35 compared with 2.3 in controls. | |
| | Metric 25: Reporting of Data | Medium | × 2 | 4 | Results were reported for all experiments and groups as mean and SD percent breaks. The number of metaphases evaluated is reported inconsistently in the study. | |
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Study Citation: K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
 Data Type: CA assay in ovine lymphocytes
 HERO ID: 194444

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--|--------|---------------------------------------|------|-------|------------------------|
| Overall Quality Determination [‡] | | High → Medium [§] | | 1.6 | |
| Extracted | | Yes | | | |

* MWF = Metric Weighting Factor.

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

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

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

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

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

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

Table 130: **In vitro** evaluation results of Sivikova et al., 2001 for MN assay in ovine lymphocytes

| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | MN assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as carbon tetrachloride, no CASRN reported. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | A commercial source (Microchem, Bratislava, Slovak Republic) was reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity 99.8% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study included a solvent (DMSO) control | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Concurrent and appropriate positive controls (mitomycin C without S9 and cyclophosphamide with S9) were used in each experiment | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided and presumed to provide more methodological details. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could affect the study results. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL) | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure durations (48hrs without and 2hrs with metabolic activation) were reported. It is presumed that these durations are acceptable for this test model system. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index). | |
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| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | MN assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | A commonly used metabolic activation system (Aroclor 1254 induced male rat S9) was used. Details on the source, method of preparation, and volume in culture were provided. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Low | × 2 | 6 | The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old) | |
| | Metric 15: Number per Group | High | × 1 | 1 | Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups | |
| | Metric 18: Sampling Adequacy | Medium | × 2 | 4 | Text reports that MN were evaluated in a total of 1000 binucleated cells per concentration but table 2 reports that 2000 binucleate cells per concentration were determined. Guidance recommends 2000. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Use of coded slides or blinded evaluations was not specified but is recommended for this endpoint. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial number of cells used per group was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis (<U+03C7>2-test) was appropriate for the outcome of interest and adequately described. Data were reported as Means with SD | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls. | |
| | Metric 24: Cytotoxicity Data | Not Rated | NA | NA | Cytotoxicity was not concurrently evaluated for this assay, however cell survival was evaluated with other assays using the same cell source and test concentrations. | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Results were reported for all experiments and groups. | |
| Overall Quality Determination [‡] | | High | | 1.4 | | |
| Extracted | | Yes | | | | |

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Study Citation: K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
 Data Type: MN assay in ovine lymphocytes
 HERO ID: 194444

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High = ≥ 1 to < 1.7 ; Medium = ≥ 1.7 to < 2.3 ; Low = ≥ 2.3 to ≤ 3.0 . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 131: **In vitro** evaluation results of Sivikova et al., 2001 for SCE assay in ovine lymphocytes

| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | SCE assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Test substance identified as carbon tetrachloride, no CASRN reported. | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | A commercial source Microchem, Bratislava, Slovak Republic) was reported. | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | Purity 99.8% | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | The study included a solvent (DMSO) control | |
| Metric 5: | Positive Controls | High | × 2 | 2 | Concurrent and appropriate positive controls (mitomycin C without S9 and cyclophosphamide with S9) were used in each experiment | |
| Metric 6: | Assay Procedures | Medium | × 1 | 2 | Assay methods were described in brief. Some details (e.g., number of lymphocytes, density at the time of exposure) were not evaluated or not reported. Specifics about slide preparations, instrumentation etc., were not included. Citations to other studies are provided and presumed to provide more methodological details. | |
| Metric 7: | Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 8: | Preparation and Storage of Test Substance | Low | × 1 | 3 | Test substance concentrations were prepared in DMSO. No details on storage were provided. No considerations for possible test substance evaporation were made (study did not indicate tests were performed in sealed containers). Due to the volatile nature of CCL4 it is expected this could effect the study results. | |
| Metric 9: | Consistency of Exposure Administration | High | × 1 | 1 | No differences in consistency of administration between groups was indicated. The final DMSO concentrations were equal (0.1%) in both control and treatment groups. | |
| Metric 10: | Reporting of Doses/Concentrations | High | × 2 | 2 | Exposure concentrations were clearly reported (2, 4, 8, and 16 ug/mL) | |
| Metric 11: | Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | The exposure durations (48 hrs without and 2 hrs with metabolic activation) were reported. It is presumed that these durations are acceptable for this test model system. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The number and spacing of exposure groups was appropriate. Justification for the high dose was provided (at least 50% reduction in mitotic index). | |
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| Study Citation: | K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | SCE assay in ovine lymphocytes | | | | | |
| HERO ID: | 194444 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | High | × 1 | 1 | A commonly used metabolic activation system (Aroclor 1254 induced male rat S9) was used. Details on the source, method of preparation, and volume in culture were provided. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | Low | × 2 | 6 | The test model (lamb primary lymphocytes) is not routinely used for the outcome of interest. Details of the source of test model were reported (Merino breed, Ovis aries L., 2-3 mo old) | |
| | Metric 15: Number per Group | High | × 1 | 1 | Independent assays were performed on cultures from two donors. Within donor replicates were not included in the study design. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was well-reported and appropriate for the outcome of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across study groups | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | The total number of metaphases evaluated for SCE (50) is adequate | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Use of coded slides or blinded evaluations were not specified. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial number of cells used per group was not reported | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on experienced disproportionate outcomes unrelated to exposure were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical analysis (ANOVA) was appropriate for the outcome of interest and adequately described. Data were reported as means with SD | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Scoring and evaluation criteria were reported. Positive outcomes were based on statistically significant changes from the controls. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | Cytotoxicity was concurrently evaluated as proliferation index | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Results were reported for all experiments and groups. | |
| Overall Quality Determination [‡] | | High | | 1.4 | | |
| Extracted | | Yes | | | | |

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Study Citation: K. Sivikova, E. Piesova, J. Dianovsky (2001). The protection of vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes Mutation Research, 494(1-2,1-2), 135-142
 Data Type: SCE assay in ovine lymphocytes
 HERO ID: 194444

| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
|--------|--------|---------------------|------|-------|------------------------|
|--------|--------|---------------------|------|-------|------------------------|

* MWF = Metric Weighting Factor.

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High = ≥ 1 to < 1.7 ; Medium = ≥ 1.7 to < 2.3 ; Low = ≥ 2.3 to ≤ 3.0 . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 132: **In vitro** evaluation results of Tafazoli et al., 1988 for MN assay

| Study Citation: | M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential <i>Mutagenesis</i> , 13(2,2), 115-126 | | | | | |
|-------------------------------------|--|---------------------|------|-------|---|--|
| Data Type: | MN assay | | | | | |
| HERO ID: | 194476 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| | Metric 1: Test Substance Identity | High | × 2 | 2 | Identified as Carbon tetrachloride and CASRN was provided. | |
| | Metric 2: Test Substance Source | High | × 1 | 1 | The commercial source was reported | |
| | Metric 3: Test Substance Purity | High | × 1 | 1 | Purity 99% | |
| Domain 2: Test Design | | | | | | |
| | Metric 4: Negative and Vehicle Controls | High | × 2 | 2 | Concurrent media-alone controls were used (no solvents were used during test preparation) | |
| | Metric 5: Positive Controls | Low | × 2 | 6 | Appropriate positive controls for conditions with and without metabolic activation were used. The data indicates that one replicate of the positive control in the presence of metabolic activation was toxic and the outcomes for this sample could not be evaluated (a separate positive control - same substance, same concentration, different donor - was able to be sampled fully - 2000 cells). The study did not discuss the reasoning for the observed cell death. | |
| | Metric 6: Assay Procedures | High | × 1 | 1 | Assay procedures were clearly described and appropriate for the outcome of interest. | |
| | Metric 7: Standards for Tests | Not Rated | NA | NA | Not applicable for the study design | |
| Domain 3: Exposure Characterization | | | | | | |
| | Metric 8: Preparation and Storage of Test Substance | Low | × 1 | 3 | Information on test substance preparation was adequately described. Methods were employed (use of sealed bottles) to prevent evaporation during the process. The duration of the test substance preparation however was lengthy (48hours, shaking at 37 degrees), and the rationale for this and the potential impact on stability was not discussed. There is further uncertainty about the stability of the test substance due to lack of DMSO as a solvent. | |
| | Metric 9: Consistency of Exposure Administration | High | × 1 | 1 | Consistent application methods are inferred from the text. | |
| | Metric 10: Reporting of Doses/Concentrations | High | × 2 | 2 | Initial Test substance concentrations (4 per donor) were reported without ambiguity. Analytical concentrations measured after the media preparation procedure were not reported. | |
| | Metric 11: Number of Exposure Groups and Concentration Spacing | High | × 2 | 2 | Exposure duration was appropriate for the outcome of interest (3hrs with activation, 48hrs without) | |
| | Metric 12: Exposure Route and Method | High | × 1 | 1 | A wide range of concentrations were tested up to a cytotoxic dose and were appropriate for the outcome of interest. | |
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| Study Citation: | M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential <i>Mutagenesis</i> , 13(2,2), 115-126 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | MN assay | | | | | |
| HERO ID: | 194476 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 13: Metabolic Activation | Low | × 1 | 3 | The study included conditions of metabolic activation (s9), however, the source and method of preparation of the S9 mix were not provided. | |
| Domain 4: Test Model | | | | | | |
| | Metric 14: Test Model | High | × 2 | 2 | The test model (primary human lymphocytes) was appropriate. Descriptive information on the source and method of isolation was provided. | |
| | Metric 15: Number per Group | High | × 1 | 1 | Two replicates (cultures at each concentration from each donor) were utilized. This is adequate according to current standards and guidelines for in vitro MN assays. | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology was appropriate for the outcome of interest | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently across exposure groups | |
| | Metric 18: Sampling Adequacy | High | × 2 | 2 | Sampling was generally adequate for the outcome of interest (2000+ cells per treatment group, except in the case of toxicity). | |
| | Metric 19: Blinding of Assessors | High | × 1 | 1 | The study reports coded slides were used. | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 20: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | It was unclear why the samples from two individual donors were treated with different doses. However, the individual donors were analyzed independently, so this is not considered to have substantially impacted results. | |
| | Metric 21: Confounding Variables in Outcomes Unrelated to Exposure | Medium | × 1 | 2 | Confounding variables on outcomes unrelated to exposure were not reported. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 22: Data Analysis | High | × 1 | 1 | Statistical methods were clearly stated and appropriate for the outcome of interest. | |
| | Metric 23: Data Interpretation | High | × 2 | 2 | Circumstances yielding a positive result were described. Positive results were based on reaching statistical significance. | |
| | Metric 24: Cytotoxicity Data | High | × 1 | 1 | The relative division index was considered a concurrent measure of cytotoxicity | |
| | Metric 25: Reporting of Data | High | × 2 | 2 | Data was adequately presented across all groups | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |
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Study Citation: M. Tafazoli, A. Baeten, P. Geerlings, M. Kirsch-Volders (1998). In vitro mutagenicity and genotoxicity study of a number of short-chain chlorinated hydrocarbons using the micronucleus test and the alkaline single cell gel electrophoresis technique (Comet assay) in human lymphocytes: a structure-activity relationship (QSAR) analysis of the genotoxic and cytotoxic potential *Mutagenesis*, 13(2,2), 115-126

Data Type: MN assay
HERO ID: 194476

| Domain | Metric | Rating [†] | MWF [*] | Score | Comments ^{††} |
|--------|--------|---------------------|------------------|-------|------------------------|
|--------|--------|---------------------|------------------|-------|------------------------|

* MWF = Metric Weighting Factor.

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High = ≥ 1 to < 1.7 ; Medium = ≥ 1.7 to < 2.3 ; Low = ≥ 2.3 to ≤ 3.0 . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

7 Developmental and Reproductive

Table 133: Animal toxicity evaluation results of Schwetz et al., 1974 for a study on inhalation developmental toxicity study in rats

| Study Citation: | Schwetz, BA; Leong, BKJ; Gehring, PJ (1974). Embryo- and fetotoxicity of inhaled carbon tetrachloride 1,1-dichloroethane and methyl ethyl ketone in rats 28(1,1), 452-464 | | | | | |
|-------------------------------------|---|---------------------|------|-------|--|--|
| Data Type: | Inhalation developmental toxicity study in rats | | | | | |
| HERO ID: | 3675473 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| Domain 1: Test Substance | | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | Reagent grade CCl4 | |
| Metric 2: | Test Substance Source | High | × 1 | 1 | Source and lot number were reported. Lot No. 9256, Burdick & Jackson Lab, Inc., Muskegon, Michigan | |
| Metric 3: | Test Substance Purity | High | × 1 | 1 | 99.9%; listed an unknown component of 0.1%; determined by gas-liquid chromatography | |
| Domain 2: Test Design | | | | | | |
| Metric 4: | Negative and Vehicle Controls | High | × 2 | 2 | Control animals for each experiment exposed concurrently to filtered room air | |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not rated/applicable for this study type. | |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups | |
| Domain 3: Exposure Characterization | | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | the method and equipment used to generate the test substance as a vapor was reported and appropriate; storage conditions were not reported | |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Details of exposure administration were reported and exposures were administered consistently across study groups | |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | nominal concentrations were reported and vapor concentration was measured analytically in the chamber and reported. | |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | 7 hr/day GD 6-15 | |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | Two vapor concentrations tested; one concentration in an initial experiment and the other in a second experiment. Each experiment had its own control group. It is not clear if these experiments were conducted concurrently. | |
| Metric 12: | Exposure Route and Method | High | × 1 | 1 | The route and method of exposure were reported and were suited to the test substance. | |
| Domain 4: Test Organism | | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Adult, Sprague Dawley female rats; starting body weight was reported | |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Medium | × 1 | 2 | Husbandry conditions were reported, but conditions were not specific for temperature, humidity, and light cycle. | |
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| Study Citation: | Schwetz, BA; Leong, BKJ; Gehring, PJ (1974). Embryo- and fetotoxicity of inhaled carbon tetrachloride 1,1-dichloroethane and methyl ethyl ketone in rats 28(1,1), 452-464 | | | | | |
|--|---|---------------------|------|-------|--|--|
| Data Type: | Inhalation developmental toxicity study in rats | | | | | |
| HERO ID: | 3675473 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 15: Number per Group | High | × 1 | 1 | The number of animals per study group was reported, appropriate for the study type | |
| Domain 5: Outcome Assessment | | | | | | |
| | Metric 16: Outcome Assessment Methodology | High | × 2 | 2 | The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest. | |
| | Metric 17: Consistency of Outcome Assessment | High | × 1 | 1 | The outcome assessment methodology addressed or reported the intended outcomes of interest | |
| | Metric 18: Sampling Adequacy | High | × 1 | 1 | Sampling for the outcomes of interest were adequate; developmental endpoints were evaluated for litters. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable; initial pathology review; no other subjective outcomes were assessed | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | The biological responses of the negative control group was adequate | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Medium | × 2 | 4 | The study reported minor differences among the study groups with respect to food consumption of dams; however, there was no effect on the conception rate or number of implantations or size of litters. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Medium | × 1 | 2 | data on attrition and/or health outcomes unrelated to exposure for each study group were not reported | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Medium | × 1 | 2 | Statistical methods were not specified in the methodology section of the paper, but are statistical tests used were specified and clear in the results tables. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Data for exposure-related findings were presented for all outcomes by exposure group | |
| Overall Quality Determination [‡] | | High | | 1.3 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High = ≥ 1 to < 1.7 ; Medium = ≥ 1.7 to < 2.3 ; Low = ≥ 2.3 to ≤ 3.0 . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

8 Mechanistic

Table 134: Animal toxicity evaluation results of Yasuda et al., 2000 study on DNA fragmentation (TUNEL assay)

| Study Citation: | M. Yasuda, T. Okabe, J. Itoh, S. Takekoshi, H. Hasegawa, H. Nagata, R. Y. Osamura, Watanabe K (2000). Differentiation of necrotic cell death with or without lysosomal activation: application of acute liver injury models induced by carbon tetrachloride (CCL4) and dimethylnitrosamine (DMN) Journal of Histochemistry and Cytochemistry, 48(10,10), 1331-1339 | | | | |
|-------------------------------------|--|---------------------|------|-------|--|
| Data Type: | DNA fragmentation (TUNEL assay) for CCl4 | | | | |
| HERO ID: | 194648 | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} |
| Domain 1: Test Substance | | | | | |
| Metric 1: | Test Substance Identity | High | × 2 | 2 | The test substance was identified by chemical name and formula. |
| Metric 2: | Test Substance Source | Low | × 1 | 3 | The source of the test substance was not identified. |
| Metric 3: | Test Substance Purity | Low | × 1 | 3 | Purity and/or grade of the test substance were not reported. |
| Domain 2: Test Design | | | | | |
| Metric 4: | Negative and Vehicle Controls | Low | × 2 | 6 | Controls were not given corn oil vehicle. |
| Metric 5: | Positive Controls | Not Rated | NA | NA | This metric is not applicable to the outcome of concern. |
| Metric 6: | Randomized Allocation | Low | × 1 | 3 | The study did not report how animals were allocated to study groups. |
| Domain 3: Exposure Characterization | | | | | |
| Metric 7: | Preparation and Storage of Test Substance | Medium | × 1 | 2 | Preparation in corn oil was described; storage was not reported. |
| Metric 8: | Consistency of Exposure Administration | High | × 1 | 1 | Gavage volume was not excessive. |
| Metric 9: | Reporting of Doses/Concentrations | High | × 2 | 2 | Information was presented for dose calculation (% in solution, mL/kg bw). |
| Metric 10: | Exposure Frequency and Duration | High | × 1 | 1 | Single gavage dose was adequate for the outcome. |
| Metric 11: | Number of Exposure Groups and Dose Spacing | Medium | × 1 | 2 | A single dose was adequate for the outcome. Dose justification was not reported. |
| Metric 12: | Exposure Route and Method | Medium | × 1 | 2 | Intraperitoneal injection is an appropriate route, but not environmentally relevant. |
| Domain 4: Test Organism | | | | | |
| Metric 13: | Test Animal Characteristics | High | × 2 | 2 | Species, strain, sex, age and starting body weight was provided. A commercial source was reported. |
| Metric 14: | Adequacy and Consistency of Animal Husbandry Conditions | Low | × 1 | 3 | Husbandry conditions were not reported. |
| Metric 15: | Number per Group | Medium | × 1 | 2 | The number of animals per group was fewer than typically used in studies of this type (2-3/group). |
| Domain 5: Outcome Assessment | | | | | |
| Metric 16: | Outcome Assessment Methodology | High | × 2 | 2 | The assessment method reported and was sensitive for the outcome of interest. |
| Metric 17: | Consistency of Outcome Assessment | High | × 1 | 1 | Outcomes were assessed consistently. |
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| Study Citation: | M. Yasuda, T. Okabe, J. Itoh, S. Takekoshi, H. Hasegawa, H. Nagata, R. Y. Osamura, Watanabe K (2000). Differentiation of necrotic cell death with or without lysosomal activation: application of acute liver injury models induced by carbon tetrachloride (CCL4) and dimethylnitrosamine (DMN) Journal of Histochemistry and Cytochemistry, 48(10,10), 1331-1339 | | | | | |
|--|--|---------------------|------|-------|--|--|
| Data Type: | DNA fragmentation (TUNEL assay) for CCl4 | | | | | |
| HERO ID: | 194648 | | | | | |
| Domain | Metric | Rating [†] | MWF* | Score | Comments ^{††} | |
| | Metric 18: Sampling Adequacy | Not Rated | NA | NA | Not applicable to the outcome of interest. | |
| | Metric 19: Blinding of Assessors | Not Rated | NA | NA | Not applicable to the outcome of interest. | |
| | Metric 20: Negative Control Response | High | × 1 | 1 | Controls responded appropriately (no positive TUNEL staining). | |
| Domain 6: Confounding / Variable Control | | | | | | |
| | Metric 21: Confounding Variables in Test Design and Procedures | Low | × 2 | 6 | Initial body weight, food/water intake were not reported for each group. | |
| | Metric 22: Health Outcomes Unrelated to Exposure | Low | × 1 | 3 | Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group. | |
| Domain 7: Data Presentation and Analysis | | | | | | |
| | Metric 23: Statistical Methods | Not Rated | NA | NA | Figures presented histology and microscopy sections. Data were not quantitative. | |
| | Metric 24: Reporting of Data | High | × 2 | 2 | Figures presented both confocal laser scanning microscopy (CLSM) and electron microscopy results. | |
| Overall Quality Determination [‡] | | Medium | | 1.8 | | |
| Extracted | | Yes | | | | |

* MWF = Metric Weighting Factor.

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0,1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High = ≥ 1 to < 1.7 ; Medium = ≥ 1.7 to < 2.3 ; Low = ≥ 2.3 to ≤ 3.0 . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.