

**Disposition of Peer Review Comments For
Five Unpublished Studies For 1,2-Dichloroethene
Toxicological Profile**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

March 2025

Peer reviewers for the five unpublished studies for the 1,2-Dichloroethene Toxicological Profile were:

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Comments provided by Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Study 1:

Confidential. 1999. "trans-1,2-Dichloroethylene and cis-1,2-dichloroethylene: inhalation median lethal concentration (LC50) study in rats."

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: The study was conducted in a manner consistent with relevant standard protocols to determine an acute lethal dose. The number of animals used was adequate for the purpose of the study. Procedures for animal care are clearly described and thorough. There are no concerns regarding animal numbers or care.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: Animal health was monitored before and during the study and the study included a control group. The possibility of competing causes of death was adequately accounted for in the study design.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: The dose groups for both cis- and trans- 1,2-dichloroethene span the range of mortality from zero to all subjects. The median lethal concentrations were approximately in the middle of the range of concentrations tested. Given these results, the number of groups and exposure concentrations were well chosen to derive the median lethal concentrations.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: Overall, the study was well designed and reported. The only aspect of the study that was not conducted under GLP protocol was preparation of the test material, which was provided by the study sponsor. The test material was well characterized, however, and I agree with the authors that this is not a significant limitation to the study.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 5: I agree with the conclusions of the authors.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Study 2:

Confidential. 2014. "In vitro mammalian cell forward gene mutation (CHO/HPRT) assay with duplicate cultures."

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: This report describes results from an in vitro mutagenicity study using a cell line. Animals (rats) were used to derive an S9 fraction to provide metabolic capability to some of the cultures. The S9 fraction was obtained commercially and no information is provided regarding the care of animals from which the S9 was derived. However, for the purpose of this study, the important consideration is the metabolic activity of the S9 fractions, which was confirmed through the use of positive control substances. Consequently, there are no concerns with respect to animal use or numbers.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: In the context of this study, cell mortality was monitored. Adjusted relative survival was not diminished by trans-1,2-dichloroethene at the highest concentration tested, so the matter of competing causes of death is moot.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: This study tested 10 concentrations, up to a concentration nearly the limit of the assay. The number of dose groups and the concentrations are sufficient to test the mutagenicity of the test substance.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: The study followed an established protocol and the methods and results are adequately reported. I found nothing that would negate the utility of the study.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.
I agree with the conclusions of the authors.

COMMENT 5: I agree with the conclusions of the authors.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Study 3:

Confidential. 2015a. "trans-1,2-Dichloroethylene: aromatase (human recombinant) assay

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: This report describes a study in which the effect of trans-1,2-dichloroethene on aromatase activity is assessed in an in vitro system. The methods follow an OPPTS protocol using a human aromatase system. No animals were used in the study.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: The endpoint examined was aromatase activity.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: The study evaluated the effects of trans- 1,2-dichloroethene at eight concentrations spanning seven orders of magnitude (10^{-10} to 10^{-3} M) in three independent runs. These conditions were sufficient to achieve the study objective of determining whether trans- 1,2-dichloroethene inhibits aromatase activity in the test system.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: The study was adequately designed and the methods, results, and conclusions are clearly described. There are no deficiencies in the study that limit its utility.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 5: I agree with the conclusions of the authors.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Study 4:

Confidential. 2015b. "Steroidogenesis (human cell line -H295R)."

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: The study evaluated the effects of trans- 1,2-dichloroethene on steroidogenesis in vitro in a human adrenocortical carcinoma cell line. No animals were used in the study.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: Cell death was evaluated using the MTT assay. Appropriate controls including two reference substances, as well as testing trans- 1,2-dichloroethene over a wide range of concentrations, were used effectively to identify any results that might result from competing causes.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: The study tested trans- 1,2-dichloroethene at seven concentrations ranging from 0.0001 to 100 μ M consistent with the U.S. EPA guideline under which the study was conducted. Per the protocol, the test was conducted in three independent experiments. These conditions are sufficient to provide a valid test of the effect of trans 1,2-dichloroethene on steroidogenesis using the model system.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: The study was adequately designed and the methods, results, and conclusions are clearly described. There are no deficiencies in the study that limit its utility.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 5: I agree with the conclusions of the authors.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Study 5:

Confidential. 2015c. "Bacterial reverse mutation assay."

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT 1: This report describes results from an in vitro mutagenicity study using several Salmonella typhimurium tester strains and an E. coli tester strain. Animals (rats) were used to derive an S9 fraction to provide metabolic capability to some of the cultures. The S9 fraction was obtained commercially and no information is provided regarding the care of animals from which the S9 was derived. The metabolic activity of the S9 fractions, which was confirmed through the use of positive control substances. There are no concerns with respect to animal use or numbers.

QUESTION: Did the study account for competing causes of death?

COMMENT 2: The principal endpoint measured in this study was revertants per plate, but toxicity was also quantified by assessing visually (under a dissecting microscope) the microcolony lawn. Positive and negative controls were used to account for competing causes of toxicity.

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT 3: An initial toxicity-mutation assay was conducted with eight different concentrations of trans- 1,2-dichloroethene ranging from 1.5 to 5000 µg/plate. A confirmatory assay was conducted with six concentrations from 15 to 5000 µg/plate. As the results from both assays were negative with respect to mutagenicity, it is important that the range of concentrations tested extended up to those producing toxicity, and they did. The number dose groups and the magnitude of the doses were sufficient to achieve the objective of the study.

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT 4: The assay was conducted in accordance with OECD and OPPTS guidelines and the methods, results, and conclusions are clearly presented. There are no deficiencies in the study that limit its utility.

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT 5: I agree with the conclusions of the authors.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Comments provided by Reviewer #2

ATSDR Charge Questions and Responses and Reviewer Comments

Study 1:

Confidential. 1999. "trans-1,2-Dichloroethylene and cis-1,2-dichloroethylene: inhalation median lethal concentration (LC50) study in rats."

COMMENT 1: Acute LC₅₀ studies were performed on both trans- and cis-1,2-dichloroethene (DCE). The study used sufficient rats per group and exposure concentrations according to OECD tg403 (5 males, 5 females per test concentration per isomer). Four test concentrations were used for each isomer. Control rats were chamber housed but not exposed to a test article. As such, the study appears to have been adequately designed. As a single 4-hour exposure, with a 14-day recovery period, competing causes of death were unlikely and not reported. According to the report, all deaths occurred during the exposures. Animal care appears to have been appropriate. There is a slight disconnect between the Summary and the documented gross observations. The Summary states that "No effects were observed in the heart, kidney, or lung in rats exposed to either isomer"; however, in Table 4 there is a dose-related trend for lung discoloration in male rats exposed to the trans isomer. Similarly, Table 5 shows a similar dose-related incidence of lung discoloration in trans-1,2-DCE-exposed female rats. The calculated LC₅₀ values seem appropriate given the data presented

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.*

Study 2:

Confidential. 2014. "In vitro mammalian cell forward gene mutation (CHO/HPRT) assay with duplicate cultures."

COMMENT 1: This report contains some confusing elements. For example, the title of the document does not indicate the substance being evaluated, and in multiple places in the document, the name of the test article is redacted. On page 6, the substance tested is indicated to be trans-1,2-dichloroethylene, CAS # 156-60-5. It is not entirely clear what is meant by a "definitive mutation assay", but the interpretation used by this reviewer is that there was a preliminary study and a full study, and that the definitive mutation assay is the latter.

In terms of the information requested, the study used no animals; the S9 was purchased from a commercial source, and it is stated that 10 concentrations of test article were used in the presence and absence of S9 in preliminary tests, and six concentrations with and without metabolic activation in the main assay. The acceptable high concentration and minimum number of concentrations tested are appropriate under the cited testing guidelines. Per OECD Guideline 476, use of duplicate cultures for each concentration is appropriate. The positive controls (EMS and BaP) performed as expected; the negative control (DMSO) showed unexplained variability in response, based on data presented in Table 2. There is a lack of transparency around the test article (why was it redacted throughout the report?), and while the data support that trans-1,2-DCE is negative in the forward mutation assay in CHO cells, I am suspicious of the amount of redacted information in the report.

RESPONSE: ATSDR thanks the Reviewer for their comments. The Reviewer did not disagree with study conclusions; therefore, no response is needed.

Study 3:

Confidential. 2015a. "trans-1,2-Dichloroethylene: aromatase (human recombinant) assay

COMMENT 1: The study described in this document is based on the Standard Evaluation Procedure Aromatase (Human Recombinant) Assay OCSPP 890.1200. The trans- isomer of 1,2-dichloroethylene (DCE) was evaluated for the potential to inhibit the conversion of androgen to estrone. As the study is performed in vitro, with microsomal protein, no animals were used. Two potential issues confound the interpretation. One is that, per the report provided, concentrations of test article were not verified using analytical methods. Also, the number of replicates of each concentration of test article and inhibitor were evaluated in each of the independent studies is not indicated. Data in Tables 4-6 include mean values and standard deviations, so it appears that $n > 1$, but it would have been desirable (and more complete) to include the $n =$ for each of the test article and inhibitor concentrations. Concentrations of the test article and inhibitor were in accordance with assay guidelines. In terms of the results, the positive control (i.e. the aromatase inhibitor 4-OH ASDN), worked as expected at the highest two or three concentrations tested. None of the concentrations of t-DCE reached the 50% inhibition benchmark for classification as an aromatase inhibitor (the lowest % activity in the presence of the test article was ~83%), indicating that the test article is not an aromatase inhibitor in this assay. Given these findings, performed in three independent assays on different days, I agree with the conclusion that the test article did not inhibit aromatase activity in the present assay system.

RESPONSE: ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.

Study 4:

Confidential. 2015b. "Steroidogenesis (human cell line -H295R)."

COMMENT 1: This study used the H295R human adrenocortical carcinoma cell line to evaluate the potential of the trans- isomer of 1,2-DCE to affect steroidogenesis; hence no animals were used. The trans isomer of 1,2-DCE was evaluated, but not the cis. The study followed US EPA Health Effects Test Guidelines, OCSPP 890.1550 for parameters such as the cell line selected, highest concentration of test article evaluated, number of concentrations tested, and data analysis. The test article was not cytotoxic at any concentration tested (MTT assay data), and positive and negative controls performed as expected (forskolin [inducer] and prochloraz [inhibitor]). I agree with the conclusion that trans 1,2-DCE was not steroidogenic in the H295R assay.

RESPONSE: ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.

Study 5:

Confidential. 2015c. "Bacterial reverse mutation assay."

COMMENT 1: This study evaluated the test article trans-1,2-DCE in the Bacterial Reverse Mutation (Ames) Assay. No mammalian species were evaluated, rather, *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, plus *E. coli* strain WP2uvrA, were used. The rat liver S9 used was

purchased from a commercial source. All test organisms were evaluated to verify their identity. Test article concentrations were used up to 5000 mg/plate, in the presence or absence of Arochlor-induced rat liver S9. Appropriate positive controls in the presence or absence of S9 were used, and the positive controls performed as expected. Based on the criteria for a positive response (at least 2-fold the number of revertants observed for the solvent control), I agree that trans 1,2-DCE did not induce a mutagenic response in the Bacterial Reverse Mutation (Ames) Assay.

RESPONSE: ATSDR thanks the Reviewer for their comments. The Reviewer agreed with study conclusions; therefore, no response is needed.