

**Disposition of Peer Review Comments For
Defense Health Agency (DHA 2022) Unpublished Study For
Chloroform Toxicological Profile**

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

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Peer reviewers for the Defense Health Agency (DHA 2022) unpublished study for the Chloroform Toxicological Profile were:

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Comments provided by Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

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

COMMENT 1: Agreed. Throughout experimentation animals were humanely euthanized by an American Veterinary Medical Association (AVMA) method approved by the Wright-Patterson Air Force Base Institutional Animal Care and Use Committee (WPAFB IACUC). Power analysis justifying animal numbers and statistical approach to data analysis are valid.

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

COMMENT 2: Exposure-induced lethality was not a study endpoint. [Focus on neurocognitive/ motor activity/ coordination effects.]

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

COMMENT 3: Dosing was performed with adequate stratification of experimental exposure parameters (2/3, 1/3, and 1/24 of LC50 for each chemical). Rats were exposed to TMS and TCM in combination using NOAEL/ LOAEL metrics. Exposure to increasing concentrations of test toxicants impaired spontaneous motor activity and/or explorative ability with a dose-dependent impact on motor coordination. Combination effects observed at lowest exposure levels impacted motor activity versus motor coordination differentially.

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

COMMENT 4: Adequately designed study with valid conclusions.

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

COMMENT 5: Data interpretation and conclusions as presented by the authors are valid potentially facilitating the definition of new exposure limits that would control the occurrence of PEs related to this specific combination toxicant exposure.

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

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

COMMENT 1:

- The study protocol was reviewed and approved by the Wright Patterson Air Force Base Institutional Animal Care and Use Committee in compliance with all federal regulations governing the protection of animals and research. Animal care is deemed to be appropriate.
- Animal numbers are appropriate and consistent with typical neurotoxicology studies. The numbers are well justified.
- It should also be noted in this section that statistical analyses were appropriate and rigorous.

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

COMMENT 2: N/A. None reported.

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

COMMENT 3:

- Both single [trimethylsilanol (TMS) and trichloromethane (TCM)/chloroform) and co-exposures were tested. Moreover, 3 doses were used to establish no observed adverse effect level or lowest observed adverse effect level (NOAEL/LOAEL). These values from single exposure studies informed co-exposure studies. In general, the Reviewer focused on chloroform studies.
- The authors used a broad concentration-response curve that covered a range of concentrations based on the LC50 for each chemical with the high, middle, and low concentrations were 2/3, 1/3, and 1/24 that of the LC50 for each chemical, respectively. Rats were exposed to TMS and TCM in combination using the no or low observed adverse effect level (NOAEL, LOAEL) determined for each chemical individually. The magnitudes are relevant. However, 3 doses are generally too few to accurately establish NOAEL, LOAEL, where ≥ 5 would be expected to be required. This does not negate the importance of the studies, but there is likely higher uncertainty in the NOAEL, LOAEL for the chosen endpoints, vs if there were more dose groups.

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

COMMENT 4: There are no concerns here. The authors should be commended for detailed technical rigor of the inhalation exposures and neurobehavioral assessments.

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

COMMENT 5:

- Primary conclusions with respect to chloroform are:
 - Dose-dependent impaired spontaneous motor activity or the animals' natural ability to explore a novel environment.
 - Dose-dependent impairment of movement coordination.
 - Post-behavioral analyses revealed that blood levels increased with their corresponding exposure concentrations.
 - There were no significant effects on the levels of neurotransmitters.
- The conclusions above are well supported with the following minor caveats. ELISA is a rather insensitive method for neurotransmitters vs UPLC-ECD or LC-MS/MS, especially for monoamines. Moreover, it is unable in many cases to distinguish between parent neurotransmitters and metabolites (not directly measured by ELISA). Thus, it is possible changes to some neurotransmitters were not captured and changes to metabolites occurred, but were not measured. The method utilized also does not detect changes to neurotransmitter release or turnover. It is also more typical to report mass of neurotransmitters relative to raw tissue mass or protein mass, vs. volume.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agreed with the primary study conclusions regarding the results of the neurobehavioral tests, which are the basis for the NOAEL/LOAEL determinations in the Toxicological Profile for Chloroform. Therefore, no response is needed.*

Comments provided by Reviewer #3

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

COMMENT 1: Yes

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

COMMENT 2: There were no deaths reported.

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

COMMENT 3: Yes, but if, as stated, the justification for the combinatorial exposure is NOAEL-based it should have been adjusted for the rotorod test. Currently, it is 1/24 LC50 based.

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 is definitely useful as it relates to motor activity and coordination effects of TMS, TCM, and their combination at lower concentrations. However, there are multiple references to cognitive/neurocognitive performance and effects; cognition was not measured in the study and any reference to effects of the test articles on cognition should be eliminated.

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

COMMENT 5: Overall, conclusions are based on the reported data as it relates to the behavioral outcomes and the blood analysis and they are in line with the data. However, conclusions also include references to effects that were not measured and, therefore, not supported (i.e. cognitive effects of TMS, TCM of their combination). Furthermore, post-behavioral neurotransmitter data are (i) very limited, (ii) largely not shown, and (iii) out of context. If such data are to be included in the study, they have to be comprehensive and relatable to both the behavioral tests used here and the reported effects of these two chemicals in the literature. Currently, there is no justifiable reasoning for analyte selection in particulate brain regions. For example, given the anesthetic properties of TMS and, especially, TCM, GABA and Glutamate should have been measured in all regions involved in motor function and coordination.

RESPONSE: *ATSDR thanks the Reviewer for their comments. The Reviewer agrees with major study conclusions regarding the results of the neurobehavioral tests, which are the basis for the NOAEL/LOAEL determinations in the Toxicological Profile for Chloroform. However, as noted in Comment 4, the Reviewer does not support some of the conclusions regarding cognitive effects based on neurobehavioral testing. ATSDR agrees with the Reviewer that neurobehavioral tests included in the study did not evaluate neurocognitive endpoints. Consistent with this, cognitive/neurocognitive performance was not discussed in the profile as none of the neurobehavioral tests in the study measured these endpoints. Findings from DHA (2022) were reported in the Toxicological Profile for Chloroform as alterations in motor activity and impaired motor coordination.*