United States
Environmental Protection Agency

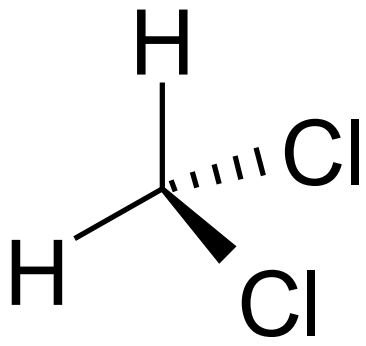
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Methylene Chloride

Systematic Review Supplemental File:

Data Quality Evaluation of Human Health Hazard Studies – Human Controlled Experiments

CASRN: 75-09-2



June 2020

Human Controlled Experiments

Acute health reference values such as the SMAC, interim AEGL 1 and 2 and California REL have been based on evidence of neurological effects in controlled human experimental studies (Putz et al., 1979; Winneke, 1974; Stewart et al., 1972) and evidence for increased formation of carboxyhemoglobin (COHb) in blood in these and other studies of humans exposed to methylene chloride (Andersen et al., 1991; Divincenzo and Kaplan, 1981; Peterson, 1978; Astrand et al., 1975; Ratney et al., 1974). EPA also identified additional human experimental studies that evaluated CNS and related effects through backwards searches of AEGL, SMAC and the California REL documents. Gamberale (1975) evaluated methylene chloride's association with reaction time, short-term memory and subjective symptoms. DiVincenzo et al. (1972) evaluated cerebral and motor functions using a wooden pegboard task. Kozena et al. (1990) evaluated reactions to weak auditory stimuli and subjective feelings before, during and after methylene chloride exposure. Winneke and Fodor (1976) described two separate experiments. In the first experiment, females conducted tasks (such as adding numbers), which were then interrupted to determine performance on another task (critical flicker frequency, or CFF). In the second experiment, females alternately performed tests of auditory vigilance and CFF. Winneke (1974) appears to describe the second experiment that is identified by Winneke and Fodor (1976), and therefore EPA did not re-evaluate this second experiment because EPA already evaluated it (as presented by Winneke (1974)).

As noted above, several studies elevated COHb levels in blood, which can serve as a biomarker for exposure to carbon monoxide (CO), in this case formed by metabolism of methylene chloride. These studies developed quantitative relationships between blood levels of COHb and various health effects. Acute health reference values for CO are based on blood levels of COHb.

EPA has not developed formal data quality criteria to evaluate these studies. Instead, EPA qualitatively evaluated individual aspects of data quality. Of the human controlled experimental studies evaluating CNS effects associated with methylene chloride (Kozena et al., 1990; Putz et al., 1979; Winneke and Fodor, 1976; Gamberale et al., 1975; Winneke, 1974; Divincenzo et al., 1972; Stewart et al., 1972), all included objective tests to measure neurological endpoints, such as visual critical flicker frequency, visual evoked response to strobe light, auditory vigilance tasks and others. Although EPA evaluated studies that examined methylene chloride directly as well as the toxicokinetic studies that determined the amount of COHb formed, EPA narrowed its focus for the risk evaluation to studies that directly tested methylene chloride's effect on CNS outcomes because there is evidence that methylene chloride exposure has a greater effect on CNS effects than just through COHb alone.

The studies differed regarding the use of blinding. Putz et al. (1979) and Kozena (1990) stated that both the volunteers and investigators were blinded to the subjects' exposures. Winneke (1974) and Winneke and Fodor (1976) used a single blind method that was not fully described; it is assumed that the volunteers (not the investigators) were blinded to their exposure status. Stewart et al. (1972) and DiVincenzo et al. (1972) did not mention whether blinding was employed, and both measured subjective symptoms. Gamberale et al. (1975) used menthol to hide the odor of methylene chloride and thus, subjects were blinded to their

methylene chloride status. These authors did not mention whether the investigators were blinded, and it is not known how the introduction of menthol affected any of the results.

Blinding was also not mentioned for the strictly toxicokinetic studies that evaluated changes in COHb (Andersen et al., 1991; Divincenzo and Kaplan, 1981; Peterson, 1978; Astrand et al., 1975; Ratney et al., 1974), although that is not expected to have had any measurable influence on the results in these studies, where outcomes consisted of measured levels of methylene chloride, CO and COHb in expired air and/or blood at various time points.

Most studies described the methods of methylene chloride atmosphere generation, and measurements (made by gas chromatography, infrared spectrophotometry or hydrocarbon analyzer) showed exposure concentrations varied little from target values for several of these studies. Comparing the inhalation method of generation against the TSCA data evaluation criteria described in *Application of Systematic Review in TSCA Risk Evaluations* (U.S. EPA, 2018), most authors used appropriate methods of test substance identification and generation of inhalation exposures (e.g., chambers with continuous metering into the room, purity information). However, Gamberale et al. (1975) used a breathing valve and gave very few details on the generation of the test concentrations; no measurements were made except for methylene chloride in alveolar air. Kozena (1990) also provided few details and used a half mask, making it more difficult to determine methylene chloride exposure concentrations. Given differences in exposure methods and limited information, these studies are difficult to compare with the other studies that measured CNS effects.

The authors across several studies chose exposure durations and concentrations to provide information useful for evaluating the relationships being investigated, including dose-dependence of findings. For the studies with toxicokinetic components, sample collection methods, timing and analytical methods were described and were appropriate.

Exposure groups were generally small, as would be expected for human experimental studies, although Winneke (1974) included group sizes as large as 18 or 20 for some exposure levels in some neurological performance tests and Kozena et al. (1990) exposed 16 males. Stewart et al. (1972) inadvertently exposed subjects to methylene chloride immediately *before* the experiment was supposed to begin, but this exposure did not result in a change in COHb levels. In the controlled exposure neurological performance studies, neurological findings are presented in relation to negative controls, but the source of the control data was not always clear. Subjects served as their own controls in several of these studies, a typical design for human experimental studies.

As a group, many of the studies provide a consistent picture of the acute neurological effects and kinetics of COHb formation associated with methylene chloride exposure in humans, which reinforces the reliability of the data from the individual studies. Most used an acceptable measure of test substance generation including analyzing the test concentrations.

Most studies describe results in sufficient detail. However, DiVincenzo et al. (1972) did not provide any details regarding their results.

Putz et al. (1979) received a medium confidence level due to use of blinding but only a single concentration. Although Winneke (1974) used multiple concentrations, the authors used a single blind method that was not well described and EPA gave this study a medium data quality rating. Because Stewart (1972) did not mention whether the investigators and

volunteers were blinded to their exposure status, EPA has low confidence in the subjective symptoms reported in this study whereas the objective measures are given a medium confidence rating.

EPA gave four studies low data quality ratings. The method of exposure, lack of measured exposure concentrations and use of menthol by Gamberale (1975) resulted in a low confidence rating. Similarly, Kozena et al. (1990) provided no details on their exposure generation and furthermore, describe use of a half mask and thus, EPA gave this study a low quality rating. Primarily because DiVincenzo et al. (1972) provided no details regarding results and limited information on whether negative controls were used, EPA gave this study a low confidence rating. Finally, the first experiment by Winneke and Fodor (1976) provided details that were too limited regarding the outcome assessment methodology and did not describe the outcomes regarding adding of numbers; therefore, EPA also gave this a low confidence rating.

The studies evaluating generation of COHb were not given confidence levels because these studies haven't been further considered for this risk evaluation.

References

Andersen, ME; Clewell, HJ, III; Gargas, ML; Macnaughton, MG; Reitz, RH; Nolan, RJ; Mckenna, MJ. (1991). Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. Toxicol Appl Pharmacol 108: 14-27.

Astrand, I; Ovrum, P; Carlsson, A. (1975). Exposure to methylene chloride: I. Its concentration in alveolar air and blood during rest and exercise and its metabolism. Scand J Work Environ Health 1: 78-94.

Divincenzo, GD; Kaplan, CJ. (1981). Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol Appl Pharmacol 59: 130-140. http://dx.doi.org/10.1016/0041-008X(81)90460-9

Divincenzo, GD; Yanno, FJ; Astill, BD. (1972). Human and canine exposures to methylene chloride vapor. Am Ind Hyg Assoc J 33: 125-135. http://dx.doi.org/10.1080/0002889728506622

Gamberale, F; Annwall, G; Hultengren, M. (1975). Exposure to methylene chloride: II. Psychological functions. Scand J Work Environ Health 1: 95-103.

Kozena, L; Frantik, E; Vodickova, A. (1990). Methylene chloride dose not impair vigilance performance at blood levels simulating limit exposure. Activitas Nervosa Superior 32: 35-37. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/ 29233

Peterson, JE. (1978). Modeling the uptake, metabolism and excretion of dichloromethane by man. Am Ind Hyg Assoc J 39: 41-47.

Putz, VR; Johnson, BL; Setzer, JV. (1979). A comparative study of the effects of carbon monoxide and methylene chloride on human performance. J Environ Pathol Toxicol 2: 97-112.

Ratney, RS; Wegman, DH; Elkins, HB. (1974). In vivo conversion of methylene chloride to carbon monoxide. Arch Environ Occup Health 28: 223-226.

Stewart, RD; Fisher, TN; Hosko, MJ; Peterson, JE; Baretta, ED; Dodd, HC. (1972). Experimental human exposure to methylene chloride. Arch Environ Occup Health 25: 342-348. http://dx.doi.org/10.1080/00039896.1972.10666184

U.S. EPA. (2018). Application of systematic review in TSCA risk evaluations. (740-P1-8001). Washington, DC: U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. https://www.epa.gov/sites/production/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf

Winneke, G. (1974). Behavioral effects of methylene chloride and carbon monoxide as assessed by sensory and psychomotor performance. In C Xintaras; BL Johnson; I De Groot (Eds.), Behavioral toxicology: Early detection of occupational hazards (pp. 130-144). Cincinnati, OH: U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health.

Winneke, G; Fodor, GG. (1976). Dichloromethane produces narcotic effect. Occup Health Saf 45: 34-35. https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/ 29075