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Project Summary

Assessment of the Mutagenic Potential of Carbon Disulfide, Carbon Tetrachloride, Dichloromethane, Ethylene Dichloride, and Methyl Bromide: A Comparative Analysis in Relation to Ethylene Dibromide

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The Reproductive Effects Assessment Group was requested by the Hazard Evaluation Division of the Office of Pesticide Programs (OPP) to prepare a mutagenicity assessment of proposed pesticide alternatives to the fumigant, ethylene dibromide. These alternatives included carbon disulfide, carbon tetrachloride, dichloromethane, ethylene dichloride, and methyl bromide. This mutagenicity assessment is to serve as a "source document" for OPP's use.

In the development of this document, the scientific literature has been inventoried, and key studies have been critically evaluated. The Environmental Mutagen, Carcinogen, and Teratogen Information Department at the Oak Ridge National Laboratory identified the published literature.

Three sections of Chapter 4 in the full report were taken from the health assessment documents prepared by the Office of Health and Environmental Assessment (OHEA) for the Office of Air Quality Planning and Standards. These sections include data evaluations of carbon tetrachloride, dichloromethane, and ethylene dichloride. The Health Assessment Document for Carbon Tetrachloride has received full administrative and peer review. The Health Assessment Documents for Dichloromethane and for Ethylene Dichloride are undergoing public review and comment and EPA Science Advisory Board review. The reader is referred to the health assessment documents (U.S. EPA, 1983a, 1984b, 1984c), if additional information is needed regarding health effects other than mutagenicity or background information such as physical-chemical properties.

This Project Summary was developed by EPA's Office of Health and Environmental Assessment, Washington, DC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

This Summary provides a brief evaluation of the mutagenic potential of five proposed alternatives to the use of the fumigant, ethylene dibromide. The alternative compounds are carbon disulfide, carbon tetrachloride, dichloromethane, ethylene dichloride, and methyl bromide, Figure 1. The evaluation involved a survey

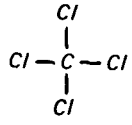
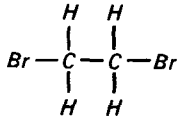
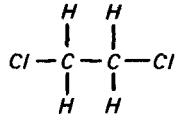
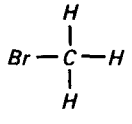
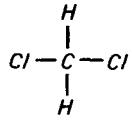
Chemical	Empirical Formula	Structure
Carbon Disulfide	CS_2	
Carbon Tetrachloride	CCl_4	
Ethylene Dibromide (1,2-Dibromoethane)	$C_2H_4Br_2$	
Ethylene Dichloride (1,2-Dichloroethane)	$C_2H_4Cl_2$	
Methyl Bromide (Bromomethane)	CH_3Br	
Methylene Chloride (Dichloromethane)	CH_2Cl_2	

Figure 1. Chemical structures of ethylene dibromide and proposed alternatives.

and critical analysis of relevant studies. A separate analysis of the mutagenicity of each proposed alternative is found in the individual sections of Chapter 4 in the full report. The evaluation of the five proposed alternative fumigants included a determination of the intrinsic mutagenic potential of each agent and its ability to reach germinal tissue in intact mammals. Ethylene dibromide is not included as a separate section in the full report because it has been evaluated previously by OPP.

A comparative analysis of mutagenicity between each of the proposed altenatives and $C_2H_4Br_2$ is presented. The spectrum of genetic damage induced by each agent is discussed and mutagenic potencies are compared whenever appropriate. Because judgments cannot be reached due to gaps in current knowledge, recommendations are made for additional studies that could be conducted to determine if a potential mutagenic risk exists.

Comparative Analysis of Ethylene Dibromide and Proposed Alternatives

This comparison considers the genetic damage that is induced by each agent and mutagenic potencies in selected tests.

Of the five proposed alternative fumigants, there is sufficient evidence on ethylene dichloride, dichloromethane, and methyl bromide, in addition to ethylene dibromide itself, to classify them as mutagens. These chemicals have been reported as positive in two or more gene mutation tests in phylogenetically different organisms, Table 1. There is also ancillary information regarding their DNA-damaging potential (e.g., SCE, DNA repair, DNA alkylation). The evidence that ethylene dichloride is a presumed mammalian mutagen is stronger than that for dichloromethane or methyl bromide because of (1) the larger number of positive tests conducted in different laboratories, (2) the suggestive evidence that ethylene dichloride causes somatic gene mutations in whole mammals, and (3) a study demonstrating the alkylation of DNA in somatic tissues of whole mammals. Although the data on the ability of these agents to cause chromosomal aberrations are limited, none of the chemicals appear to be strong clastogens. It is uncertain whether these agents produce a similar array of other types of genetic damage (e.g., nondisjunction, SCEs, mitotic recombination), because they have not all been sufficiently evaluated for the induction of other types of genetic alterations. Furthermore, it is uncertain whether the proposed alternatives reach and interact with mammalian germ-cell DNA, but ethylene dibromide is known to do so and this is presumed to be a human germ-cell mutagen. Ethylene dichloride, methyl bromide, and dichloromethane are positive in the Drosophila sex-linked recessive lethal test. This test organism has germ-cell stages analogous to those in mammals and provides some information regarding germ-cell risk in intact animals. Although the data bases are not equally complete for each of the compounds, none of the proposed alternatives appear to be as mutagenic as ethylene dibromide when results from similar tests are compared.

The mutagenic potential of the other two alternatives, carbon disulfide and carbon tetrachloride, could not be judged because of insufficient information. The available studies suggest, however, that if they are mutagenic, they are weakly so. This conclusion does not necessarily apply to chromosome non-disjunction because carbon tetrachloride has not been evaluated for its ability to disrupt spindle structures or function, and inadequate evidence is available for the induction of numerical chromosomal aberrations by carbon disulfide. The reader is referred to Chapter 4 of the full report for a critical analysis of the data pertaining to the mutagenicity of these five proposed alternative fumigants and for a detailed summary for each chemical.

Mutagenic Potencies

The mutagenic potencies of each of the five proposed alternative fumigants were compared with those of ethylene dibromide using results from the Salmonella assay. This test was the only one in which all chemicals have been evaluated. Several criteria were imposed to select appropriate experiments for this analysis. Only experiments using the desiccator procedure were included because all of the chemicals are volatile, and testing in sealed containers is more appropriate than in the standard plate assay in which the volatile test material evaporates and escapes. In addition, results were considered only if there were at least two nonzero dose points; spontaneous counts were reported, and revertant data were given in the report. Results on tested

Table 1. *Qualitative Comparison of the Mutagenicity of Ethylene Dibromide and Proposed Alternatives*

	Gene Mutation						Clastogenicity		Numerical Chromosome Mutation	Other Indicators of DNA Damage	DNA Binding
Chemical	Bacteria	Fungi	Higher Plants	Drosophila	Mammalian Cells	Whole Mammals	In Vitro	In Vivo			
Carbon disulfide	-(1) I(3)			I(2)				W(1) -(2)	I(2)	I(1), UDS	
Carbon tetrachloride	W(1) I(4) -(2)	W(1)					-(1)			W(1), YMR -(3), UDS	+(2)
Dichloromethane	+(12) I(2)[1]	+(1)		+(1) I(1)	I(1)		+(1)	W(1) -(1)		(I), YMR (+), YMR W(2), SCE -(2), UDS	
Ethylene dibromide	+(5) -(4)	+(3)	+(3)	+(3)	+(2)		+(1)	+(2)[a] -(2)		+(1), YMR +(1), UDS -(1), UDS +(1), Pol +(1), SCE	+(1)
Ethylene dichloride	W(7)[b] I(3)		+(1) I(1)	+(4)	+(2)	W(1)		-(2)	+(1)[c] W(1)[c]	I(1), UDS I(1), Pol	+(1)
Methyl bromide	+(4)			+(1) I(1)	+(1)			I(2)		I(2), UDS	+(1)

Information on ethylene dibromide is based on OPP documents (U.S. EPA, 1983a; Mauer, 1979; Lee, 1980).
"+" designates a positive result; "-" a negative result; "I" an inconclusive study; "W" a weak, borderline, or suggestive result.
Numbers of studies are indicated in parentheses.
UDS = unscheduled DNA synthesis; YMR = yeast mitotic recombination; SCE = sister chromatid exchange; Pol = bacteria.
[a]*Plants only.*
[b]*Stronger response with an S9 metabolic activation system.*
[c]*Drosophila only.*

strain TA100 in the presence or absence of metabolic activation were used because this was the only strain for which data were available on all compounds. A simple linear regression analysis was used on the linear portion of the dose-responses; linear regression calculations with correlation coefficients less than 0.90 were not accepted.

It was clear from these tests that ethylene dibromide is a much more potent mutagen than the corresponding chlorinated compounds and more mutagenic than methyl bromide based on structural-activity relationships. Ethylene dibromide is a bi-functional agent, and thus more biologically reactive. Carbon tetrachloride and carbon disulfide are predominantly negative in bacterial tests.

Potencies were also examined in the Drosophila sex-linked recessive lethal test. Data were available for ethylene dibromide, ethylene dichloride, dichloromethane, and methyl bromide. Although certain germ-cell stages appear to be more sensitive than others, the total lethal frequencies were compared and it was found that ethylene dibromide is a more potent mutagen than the alternatives, whether data from inhalation or feeding experiments are compared. It is uncertain if ethylene dichloride is more active than methyl bromide. There is some overlap of the lethal frequencies per unit of exposure for these alternatives. Different strains of Drosophila were used, which could account for differences in lethal frequencies. Although carbon disulfide has been reported as negative, the possibility of weak effects cannot be excluded. In feeding experiments, ethylene dichloride seems to be more active than dichloromethane. However, these experiments were conducted in different laboratories, and factors such as stocks of Drosophila and solvents differed; these variations could contribute observed differences among the alternatives in lethal frequencies. Nevertheless, in their totality, the data show that ethylene dibromide is more mutagenic than the proposed alternatives in the Drosophila sex-linked recessive lethal test. The Drosophila results are therefore consistent with those in Salmonella.

The mutagenicity of the alternative compounds in cultured mammalian cells cannot readily be compared to that of ethylene dibromide because test results are based on different cell lines and different loci. However, in one study using human lymphoblasts and another study using Chinese hamster ovary cells, ethylene dibromide was a more potent mutagen than ethylene dichloride.

Ethylene dibromide, ethylene dichloride, and methyl bromide have been examined for DNA adduct formation in whole mammals after inhalation exposure. Although there is DNA binding data with carbon tetrachloride, it is derived from intraperitoneal injection experiments and therefore were not used in comparison with the other compounds. Liver was the only organ in which DNA

alkylations could be compared for the three chemicals. It was found in these experiments that methyl bromide binds DNA to a much lesser extent than does ethylene dibromide; approximately five orders of magnitude difference were observed.

Ethylene dichloride appears to bind to DNA to a greater extent than methyl bromide but to a lesser extent than ethylene dibromide. It should be cautioned, however, that the measurements for ethylene dichloride were derived from a different rodent species (rat) than those for ethylene dibromide and methyl bromide (mouse). Although there were two orders of magnitude difference in the alkylations, a species difference could conceivably account for the amount of binding.

Given the available data, few conclusions can be drawn about DNA alkylation by methyl bromide and ethylene dichloride, except that both agents interact with DNA. Methyl bromide appears to do so to a lesser extent than does ethylene dibromide. Information from experiments that involve measurements at different time intervals to determine the stability of various DNA adducts formed by these compounds would be a valuable addition to current knowledge. If stable adducts are formed in testicular DNA, which is a target tissue for heritable risk, then the induced genetic damage could accumulate during the cellular life cycle of the gonial cells. The gonia are an important cell type relevant for human genetic risk assessment. In the case of ethylene dibromide, it is known that adducts are formed in testicular DNA. Data regarding the degree of alkylation is more useful if it includes information on the type of adducts formed and the stability of these adducts. Such information is needed for ethylene dichloride, methyl bromide, and dichloromethane in germinal tissue of intact mammals.

Conclusions and Recommendations

The five proposed alternatives do not appear to be as mutagenic as ethylene dibromide. Two alternatives, carbon tetrachloride and carbon disulfide, have been primarily negative in mutagenicity testing, however it cannot be stated that they do not pose a mutagenic risk because the available information is limited and sometimes inadequate. Additional testing would be necessary for them to be classified. It should be noted that even if these agents do not pose a mutagenic hazard, they do pose other health hazards; for example, carbon disulfide is extremely toxic and carbon tetrachloride is extremely toxic and carcinogenic in mice and rats.

The alternative compounds that are mutagenic in several short-term gene mutation assays are ethylene dichloride, dichloromethane, and methyl bromide. It cannot be concluded that one of these agents is more mutagenic than the other because of limited data. It does appear, however, that these agents are not strong mutagens, because rather large, and often toxic, doses are required to elicit mutagenic responses. Delineation of differences in mutagenic activity among these agents will require dose-response data that are generated in the same laboratory so as to minimize technical and biological variation. The proposed alternatives are all volatile chemicals and precautions are therefore essential to prevent excessive evaporation of test material. Several different assay systems, including mammalian systems, should be used to determine a rank order for mutagenic potency. If these experiments were coupled with molecular dosimetry, the relationship of mutation frequency could be compared to target dose rather than to exposure. If a similar rank order of potency is observed in different species, it might be reasonable to assume that a similar ranking may exist in humans. After these determinations, whole mammal germ-cell studies would have to be conducted to estimate heritable risk.

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The complete report, entitled "Assessment of the Mutagenic Potential of Carbon Disulfide, Carbon Tetrachloride, Dichloromethane, Ethylene Dichloride, and Methyl Bromide: A Comparative Analysis in Relation to Ethylene Dibromide," (Order No. PB 85-241 800/AS; Cost: $16.00, subject to change) will be available only from:

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