CHLOROMETHANE

DRAFT SUPPORT DOCUMENT FOR TSCA SECTION 4 TEST RULE

Test Rules Development Branch
Assessment Division
Office of Testing and Evaluation
Office of Toxic Substances
Environmental Protection Agency

TABLE OF CONTENTS

Summary, Proposed Testing and Justification.....1
A. Health Effects.....1
B. Exposure.....21
C. Material to Be Tested.....21

I. Identity of Chloromethane.....22

II. Exposure Aspects.....24

A. General.....24
B. Direct Exposure.....26
C. Environmental Exposure.....31
D. Existing and Proposed Guidelines and Standards for Chloromethane.....35

III. Health Effects.....38

A. Systemic Effects.....38
B. Neurotoxicity.....49
C. Mutagenicity.....58
D. Oncogenicity.....66
E. Teratogenicity.....74
F. Metabolism.....76

IV. Current and Planned Testing.....89

References.....98

Summary, Proposed Testing and Justification

In its first report to the EPA, October 1977, the Interagency Testing Committee (ITC) recommended that chloromethane be given priority consideration for the development of testing requirements under Section 4 of the Toxic Substances Control Act (ITC 1978). Specifically, the ITC recommended that chloromethane be tested for its carcinogenicity, mutagenicity, teratogenicity, and other chronic effects. With regard to chronic effects, the ITC expressed particular concern for chloromethane's effects on the central nervous system, liver, kidney, bone marrow, and the cardiovascular system.

A. Health Effects

On the basis of information presented in the following sections, the EPA is proposing that chloromethane be tested for its potential chronic neurologic and behavioral effects, mutagenicity, oncogenicity, and teratogenicity, both morphologic and behavioral. This document is in support of the EPA's proposed test rules requiring such testing. As soon as test standards have been developed and proposed covering the necessary neurotoxicity and behavioral teratogenesis effects, these will be proposed in a subsequent rulemaking.

1. Chronic Neurotoxicity

a. Summary and Findings

Several investigators have detailed the permanent neurobehavioral effects of long-term exposure to chloromethane.

Klimkova-Deutschova (1957), Langauer-Lewowicka et al. (1974), and Milkov et al. (1965) are representative of studies that deal with groups of workers in the chloromethane industry (either direct or indirect) who exhibited chronic neurologic or behavioral changes from long-term exposure, with no known high-level acute exposures. In a slightly different type of study, Repko et al. (1977) found significant decrements in complex math tasks, increases in resting tremor, and increases in the latency to visual stimuli in a group of chloromethane-exposed workers, while testing in the workplace. The EPA feels that while these studies suggest that long-term exposure to chloromethane may pose an unreasonable risk, they are inadequate to determine the extent of that risk.

Animal studies have also been done, but many problems in evaluating these studies occur. Smith and von Oettingen (1947a,b) tested chloromethane in several species of animals and concluded that 300 ppm had "no apparent effect in 64 weeks of exposure" on any species tested, but that (a) the effects of 500 ppm in dogs and monkeys had much in common with symptoms described for humans and, therefore, that (b) "then it is evident that daily exposures to concentrations of 500 ppm are extremely dangerous even for a period of two weeks or less".

More recent animal studies of chronic exposure have produced suggestive evidence of functional and pathologic effects after a shorter duration of exposure at lower concentrations. A Russian study (Yevtushenko 1966), which the author cited as one basis for the 1965 Soviet TLV of 2.5 ppm, reported effects in rats and

rabbits at low levels in both acute and chronic exposures. This study reported an increase in the time to acquire a conditioned response in rats after 4 hours of exposure to as little as 114 ppm. Furthermore, after six months of exposure to 20 ppm rats showed behavioral deficits. Pathologic changes in rabbits exposed in the same experiment (Belova and Yevtushenko 1967) occurred throughout the brain as well as in the eye at the low dose.

b. Testing

One specific neurobehavioral effect of concern has been identified for testing, namely, chronic effects on function and morphology of the nervous system. While the Agency is not prepared at this time to propose complete standards for the conduct of such testing, set forth below are current views on the proposed testing, and related issues relevant to the development of these standards. Comments are solicited from all sectors on the appropriateness and conduct of the suggested testing.

Based on the recent controlled laboratory studies of Putz et al. (1979) and Stewart et al. (1977), and the worker study of Repko et al. (1977), it appears that changes in complex cognitive functions, and visual function as measured by behavioral tasks, may be the most sensitive human indicators of exposure to chloromethane. The report on exposed workers by Klimkova-Deutschova (1957) as well as the 13 year follow-up study of exposed fishermen by Gudmundsson (1977) suggest that chloromethane intoxication may induce damage that involve the cranial

nerves or other structures controlling the eye, pyramidal and extrapyramidal neurologic signs, a reduced tolerance to alcohol, fatigue, and depression.

The EPA is considering proposing animal studies to determine no-effect levels for neurobehavioral effects of chronic exposure. Among the variables to be determined are choice of species, length of test, days per week exposed, type of exposure and type of testing. It has not yet been determined that in this case the most sensitive human indicators of chloromethane neurotoxicity would necessarily be those tested by choice in an animal model. The EPA is asking for public comment on these issues in an ANPRM.

The choice of species for animal testing will involve several considerations. First, Smith and von Oettingen (1947a) have suggested that dogs and monkeys are more sensitive than the other species they tested, and that effects in these species most resemble human intoxication. The inappropriateness of rats as a test species is suggested by the same authors' failure to observe any overt effects in rats but not other mammalian species exposed to 500 ppm. On the other hand, Yevtushenko (1966) reported behavioral effects from both acute and chronic exposure to low levels in rats; the apparent discrepancy may be due to their use of quantified behavioral testing as compared to the presumably less objective observational techniques of Smith and von Oettingen (1947b). However, the reports of neither study are adequate to determine if this is, in fact, the case. The ocular conjunctivitis observed by Belova and Yevtushenko (1967) in rabbits and more recently by CIIT (1979a) in mice but not rats

suggests that rats are less sensitive with respect to ocular irritation as well.

The Agency is also considering the appropriateness of and the best means of defining adequate post-exposure testing of subjects from all exposed groups to assess the severity of delayed effects, if any, and the persistence of any observed effects. If exposure in chronic testing is noncontinuous, these effects could be assessed in part during chronic exposure studies prior to the beginning of daily or weekly exposure.

In addition, the Agency is considering whether testing for abuse potential, interaction with ethanol and/or a mixed schedule of exposures (long-term low-level plus acute high-level) would be appropriate additions to the requirements. Details are presented in an ANPRM.

2. Mutagenicity

a. Summary and Findings

There is evidence from bacteria and higher plants that chloromethane is capable of causing both gene mutations and chromosomal aberrations. In bacteria, chloromethane is a direct-acting mutagen capable of inducing base pair substitutions in the DNA of *S. typhimurium* strains TA 1535 and TA 100 (Andrews et al. 1976, DuPont 1978, Simmon 1978). In *Tradescantia* pollen grains, chloromethane is more effective than ethylene oxide in inducing chromatid breakage (Smith and Lotfy 1954). Although this information indicates that exposure to chloromethane may present an unreasonable risk of mutation to humans, it is insufficient by

itself to assess chloromethane's risk as a potential human mutagen. Therefore, the EPA is requiring more extensive testing to determine if chloromethane may be so classified and to generate the information necessary to perform a mutagenicity hazard assessment on chloromethane.

b. Testing

In recent years, mutagenicity experts have discussed, and provided guidance on, hazard estimation procedures for determining if a chemical is a potential human mutagen.

Four major reports on the hazards of environmental mutagens were issued between 1975 and 1979 (Drake 1975, Flamm 1977a,b, McElheny and Abrahamson 1979). In 1978 the Office of Pesticide Programs proposed Guidelines for Registering Pesticides in the U.S. (OPP 1978) and a report entitled "Mutagenicity Guidelines" is being prepared by Dr. W.G. Flamm for the Office of Health Effects Assessment describing mutagenicity risk assessment procedures for use by the Agency (Flamm 1979).

The reports agree that to perform a mutagenicity hazard estimation for humans, scientists must first demonstrate that a substance and/or its metabolite(s) does or does not cause heritable gene or chromosomal mutations (the two classes of mutagenic damage which have been shown to be responsible for a portion of human genetic disease) and whether or not the mutagenically active form can reach the genetically significant target molecules in mammalian germinal tissue.

A discussion of the principles and practices of mutagenicity testing in terms easily understood by persons unfamiliar with mutagenicity is presented in the EPA's booklet "Short-Term Tests for Carcinogens, Mutagens and other Genetoxic Agents" (EPA 1979a).

The rationale for utilizing mutagenicity data which are not derived from humans (all present data) has been previously detailed (OPP 1978) and is based on an extensive body of knowledge in the field of genetics. The following points are essential to such a rationale and are generally accepted by experts in the field of mutagenesis (see e.g., Drake 1975, Flamm 1977a,b, McElheny and Abrahamsom 1979). They are:

(1) All organisms (except for a few viruses) have DNA as the genetic material which is basic for survival and reproduction;

(2) The DNA code is the same in all organisms;

(3) The cellular machinery for decoding the information stored in the DNA code is similar among all organisms;

(4) Eukaryotic organisms contain nuclei in their cells, and their DNA is associated with protein to form complex bodies called chromosomes. Prokaryotic organisms lack nuclei, and their chromosome structure differs from that of eukaryotic organisms;

(5) Unless there is a mutational event, the information in DNA is faithfully replicated in each cell generation in unicellular organisms and in somatic and germ cells of multicellular organisms;

(6) DNA can be altered by chemicals. If this damage is repaired properly there is no mutation. If it is repaired with error or not repaired prior to replication of DNA, mutation can result. A single lesion in DNA may lead to a mutation;

(7) Point mutations usually involve changes in the bases of the DNA chain: the replacement of one purine or pyrimidine DNA unit by another is called base pair substitution: insertion or deletion of a base pair into the DNA chain is called a frameshift mutation;

(8) Breaks in DNA may lead to structural chromosomal aberrations;

(9) Disturbances in the distribution of individual chromosomes or chromosome sets can occur during cell division and result in numerical chromosomal aberrations; and

(10) Mutations are generally considered to be deleterious in reference to the normal environment for an organism and to result in decreased survival and reproduction.

Although not all mutations are deleterious (e.g., the Ames test measures a mutation which is advantageous to the organism), it is impossible to tell if any alteration in the genome would be good, bad, or of no importance.

Given the ubiquitous nature of DNA as the genetic material, the universality of the genetic code, and the similarity in response of genes and chromosomes of various lifeforms, a rationale for using the results from different test systems develops. Humans, as well as bacteria, fungi, and higher eukaryotes suffers DNA damage and gene mutations; man, as well as other eukaryotes, shows structural and numerical chromosomal aberrations. For these reasons, cells of any species may be used to detect genetic changes and to predict genetic change or damage in other species.

There are two tests each of which measures one of the genetic endpoints (gene mutation or chromosomal aberration) and the ability of a mutagenically active form of a chemical to reach germinal tissue. These tests are the mouse specific locus test and the heritable translocation test. Both of these tests are performed in mice. The mouse specific locus test detects gene mutations; the heritable translocation test detects chromosomal aberrations as its genetic endpoint.

The EPA is not requiring a mouse specific locus test for chloromethane for the following reasons. Chloromethane is a known

alkylating agent with the demonstrated capability to react with cellular nucleophiles. The rodent alkylation and Drosophila sperm alkylation tests discussed below were both designed to detect the ability of alkylating agents to react with the DNA of germinal tissue. These tests are extremely sensitive. Sega et al. (1974) have shown DNA alkylation in mouse sperm at levels below those detected in the mouse specific locus test with ethyl methanesulfonate. Aaron and Lee (1978) reported a genetic alkylation in Drosophila sperm at levels just over 4X the spontaneous mutation rate in this system. Detection of genetic events at this level in the mouse has not been reported. For alkylating agents, then, DNA alkylation may possess superior sensitivity to the mouse specific locus test, and is appropriate, therefore, for measuring the ability of chloromethane to interact with the DNA of germinal tissue. For these reasons, the EPA believes a mouse specific locus test on chloromethane is not warranted.

(1) Test Requirements for Determining Gene Mutation

Tests will be required to demonstrate the potential of chloromethane to induce heritable gene mutations in a higher organism. In addition, the ability of chloromethane to interact with mammalian germinal tissue will be determined. The tests to be performed include:

(a) The sex-linked recessive lethal test (DSRL) in Drosophila melanogaster;

(b) DNA alkylation in rodent (RA) and Drosophila (DA) sperm cells;

(c) Gene mutation in mammalian cell culture (MCC);

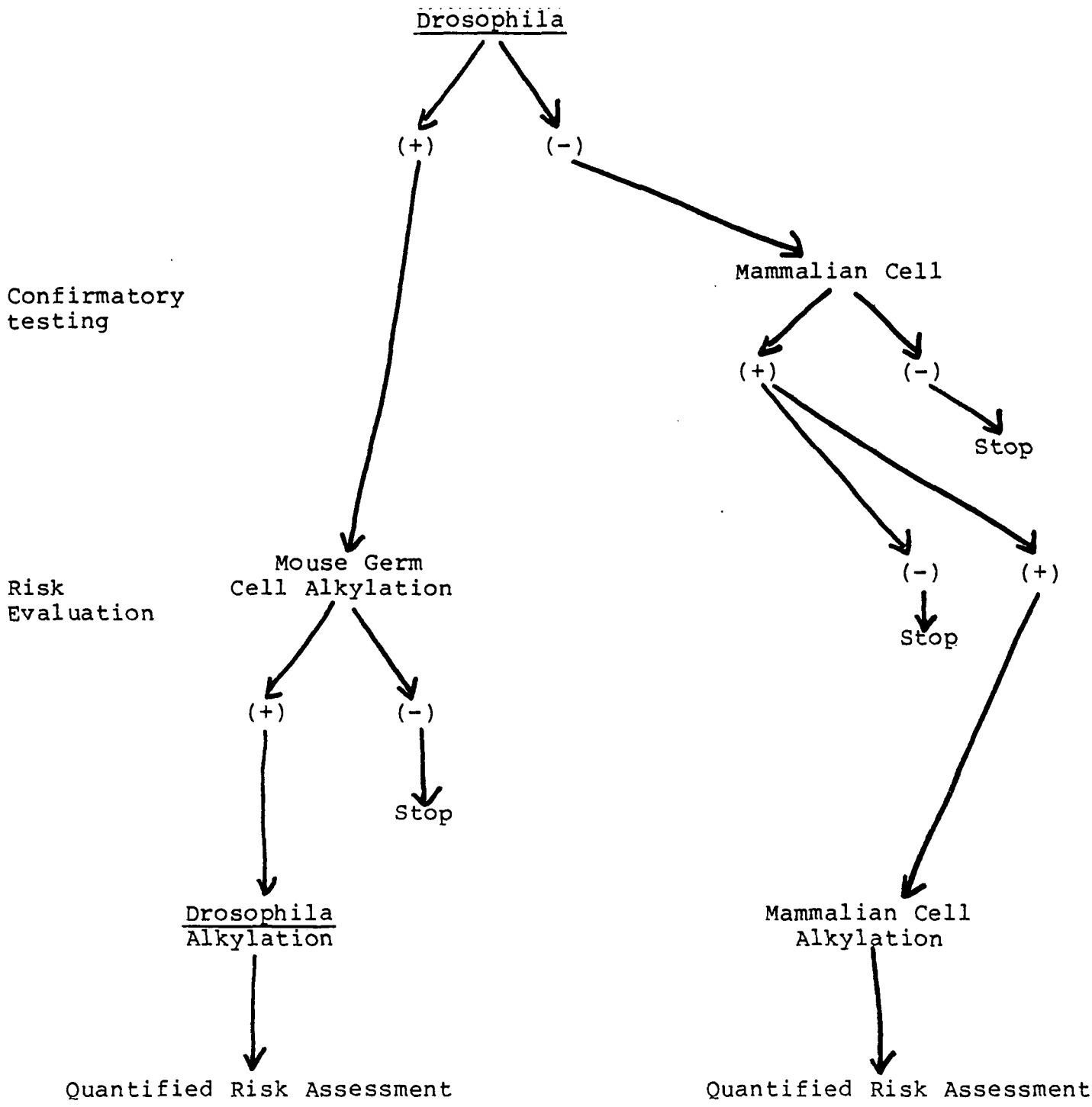
(d) DNA alkylation in mammalian cell culture (MCCA).

These tests will (1) generate information necessary to classify chloromethane as a potential human mutagen and to perform a mutagenicity hazard assessment; or (2) determine that chloromethane does not appear to induce heritable gene mutations in mammals and, therefore, cannot be classified as a potential human mutagen. (See Chart on following page.)

The sex-linked recessive lethal test in Drosophila will be the first test to be performed. If the DSRL is positive the next test to be performed will be rodent sperm alkylation. A positive rodent sperm alkylation will be followed by a determination of DNA alkylation in Drosophila sperm. If the results of the rodent sperm alkylation test are negative, no further testing will be required at this time.

A negative DSRL in Drosophila will be followed by a test for gene mutations in cultured mammalian cells. A positive mammalian cell assay will be followed by a determination of rodent sperm alkylation. A positive rodent sperm alkylation will be followed by a determination of the extent of DNA alkylation in mammalian

CHLOROMETHANE
Gene Mutation Testing Scheme



cell culture. No further testing will be required if chloromethane is inactive in either the mammalian cell culture or rodent sperm alkylation assays.

The results of these tests will be used to perform a risk assessment of the gene mutation hazard to the human genome.

The EPA believes that as a matter of policy, further testing for gene mutations of a chemical that fails to induce heritable sex-linked recessive lethal mutations in Drosophila or gene mutations in mammalian cells in culture should not generally be undertaken. Should chloromethane, therefore, be negative in these assays, the EPA would require no further testing for gene mutations at this time.

In the absence of evidence that a chemical and/or its reactive metabolites can reach mammalian germinal tissue and once there interact with germinal tissue DNA (the rodent sperm alkylation assay), evidence to classify the chemical as a potential human mutagen is lacking and hazard assessment is unwarranted.

To determine the mutagenic risk to humans of a chemical such as chloromethane, it is necessary to estimate the dose that human germ cells will receive. This estimation can be made from metabolic fate studies in mammals where the alkyl group is labeled with a radionuclide and the alkylations per nucleotide of DNA are determined, using methods developed by Sega et al. (1974). The rodent sperm alkylation test gives a measure of mammalian body exposure to a mutagen and relates to mammalian gonadal dose.

To estimate genetic risk from a dose measured as alkylations per nucleotide (AN) in the germ cells, it is necessary to determine a dose (AN)-response relation in an experimental system that can be thoroughly analyzed genetically for mutations induced in the different germ cell stages. The required experimental data is obtained from the relationship of dose to mutagenic response in D. melanogaster using several of the genetic tests available in this species. To determine the mutational risk of a compound present in low levels in our environment it is necessary to determine the shape of the dose-response curve from the Drosophila experiments and the exposure-dose curve from the metabolic fate studies. Methods for determining the dose of alkylating agents measured as alkylation per DNA nucleotide in the germ cells of Drosophila melanogaster have been developed (Lee 1978, Aaron and Lee 1978). Therefore a determination of the mutation frequency induced by a given alkylation level can be made.

This battery of tests has as its general justification the assumptions regarding mutagenicity testing which were presented above. Specifically, the Drosophila sex-linked recessive lethal (DSRL) test detects heritable gene mutations in an insect. The rodent sperm alkylation test measures the ability of a given chemical and/or its metabolites to reach and to interact with the DNA of mammalian germinal tissue. As described above, this information can be used to estimate mutational risk to humans from exposure to chloromethane.

It is possible that an agent capable of producing heritable gene mutations in mammals would not be detected in Drosophila because of species differences between insects and mammals. In the case of chloromethane, a DSRL test is being required before a test for mutations in mammalian cell culture (MCC). It is believed that because of the volatile nature of chloromethane, exposure of Drosophila will be technically easier to achieve than tests in MCC. Both procedures are feasible, however, and a negative DSLR will be followed by a test for mutations in MCC. A positive result in mammalian cell culture together with a demonstration of the ability of the chemical to reach and interact with mammalian germinal tissue DNA is sufficient evidence to classify a chemical as a potential human mutagen. Data gained from alkylation of the DNA in mammalian cell cultures (Aaron et al. In press) may be used to estimate risk to man in a manner analogous to that described above for Drosophila.

(2) Test Requirements for Chromosomal Aberrations

In addition, the EPA is requiring that chloromethane be tested for its potential for causing chromosomal aberrations, starting with a dominant lethal assay in rodents. If chloromethane is inactive in the dominant lethal assay, no further testing will be required. A positive dominant lethal assay will be followed by a heritable translocation assay. A quantified chromosomal aberration hazard assessment will be performed if chloromethane is positive in the heritable translocation assay. This scheme is illustrated by the following diagram:

Dominant lethal assay	(+) Heritable Translocation	(+) Quantified Hazard Assessment
(-) No further testing	(-)	

The dominant lethal test is used to trigger a heritable translocation test because all chemicals positive in the heritable translocation test are positive in the dominant lethal test. However, not all chemicals positive in the dominant lethal are positive in the heritable translocation test (Flamm 1977b). Therefore, a positive dominant lethal result triggers a heritable translocation. Hazard assessment is performed on the basis of results of the heritable translocation test.

(3) Other

In this series of required tests, the EPA is not specifying test details such as mammalian cell line, or type of mutation in the mammalian cell culture assay, or species to be used in the dominant lethal assay. Protocols to be followed for all tests except DNA alkylation have been published by the EPA (EPA 1979b). Protocols to be followed for sperm alkylation, mammalian cell culture alkylation and *Drosophila* alkylation have been published by Sega et al. (1974), Aaron et al. (1980) and Aaron and Lee (1978).

3. Oncogenicity

a. Summary and Findings

After reviewing the evidence available on the oncogenic potential of chloromethanes, the EPA finds that chloromethane may be an oncogen. Although there is no direct evidence in humans or in animals that chloromethane is an oncogen, various indirect evidence indicates that it has oncogenic potential. The indirect evidence is summarized as follows:

a. chloromethane is a mutagen: (1) inducing gene mutations and (2) causing chromosomal aberrations.

b. chloromethane is a direct alkylating agent which is known to alkylate human and animal tissues; and

c. chloromethane is a close structural analogue of two potential human oncogens, carbon tetrachloride and chloroform, and of a suspect human oncogen, iodomethane. Another suspect analogue with weaker evidence of potential oncogenicity is dichloromethane. Chloromethane is metabolized to yet another possible oncogen, formaldehyde.

b. Testing

The EPA will require that a 2-year oncogenicity study be undertaken to determine the oncogenicity potential of chloromethane in animals (EPA 1979c). The justification for such

a requirement is two-fold: 1) the available information indicating that chloromethane possesses oncogenic potential is of sufficient strength to make a finding that the chemical may represent an unreasonable risk; and 2) the finding that the only study available (see Section IV) to evaluate the oncogenic potential of chloromethane in animals is inadequate for EPA purposes, if the results are negative.

4. Teratogenicity

a. Summary and Findings

Because of the biological activity of chloromethane in adults, its probable accessibility to the fetus, and the embryo-fetal effects of closely related compounds, the EPA believes that chloromethane may present an unreasonable risk of teratogenicity. With regard to the teratogenic potential of chloromethane, the EPA is concerned with the danger of both structural malformations and behavioral alterations.

b. Testing

Standards for the development of data on morphologic teratogenic effects have been proposed (EPA 1979b). These standards relate to the development of data on anatomical abnormalities. The EPA believes that these standards are appropriate for the testing of chloromethane for the induction of these effects.

The requirements for the testing of chloromethane for teratogenic effects is based on the need to develop data for

assessing this aspect of possible health effects under the Toxic Substances Control Act (TSCA). This need has been developed with the knowledge that certain toxic chemicals produce developmental deviations (Wilson and Fraser 1977). These developmental deviations may take the form of death, malformation, growth retardation and/or functional disorder. It is an underlying assumption that production of these effects in humans constitute unreasonable risks.

The use of animal testing to evaluate teratogenic potential in humans has been accepted world-wide (FDA 1970, MHW 1976, WHO 1967). In addition, the value of animal testing is supported by the observation that all substances known to be teratogenic to man can be shown to be teratogenic in laboratory animals (WHO 1967). Although the full theoretical relationship between animal test data and human teratogenic potential has yet to be established, it is generally accepted that substances shown to be teratogenic in animals may be teratogenic to man under appropriate conditions of dosage and timing. Positive data of this type from animal studies will be considered by the EPA as substantial evidence for teratogenic potential in man, surpassed only by human epidemiologic studies and case histories.

In addition, the EPA is proposing to include an evaluation of neurologic/behavioral abnormalities and of the acquisition of developmental landmarks in its development of data on the possible teratogenicity of chloromethane (see, e.g., Vorhees et al. 1979b). Since no standards for the development of this type of

data have been proposed, this topic will be included in the ANPRM for chloromethane, and will be subject to public comment.

In addition to routine signs of physical development that may reflect toxicity (e.g., body weight), the proposed testing should include specific tests to assess in the offspring known effects of chloromethane in adults. Acquisition of a conditioned reflex was reported as a sensitive endpoint by Yevtushenko (1966). Neurologic impairment of motor function in humans and other mammals has been reported (see, e.g., Klimkova-Deutschova 1957, Smith and von Oettingen 1947b) and impairment of visual functions in humans (see, e.g., Langauer-Lewowicka et al. 1974). These three types of endpoints should be considered as well as thorough neuropathology.

5. Other Chronic Toxicity

Although the Interagency Testing Committee (ITC) recommended testing to determine chronic effects on the liver, kidneys, bone marrow, and cardiovascular system, the EPA is not proposing requiring such studies. Results available from previous studies, especially those of Smith and von Oettingen (1947a,b), Smith (1947), Dunn and Smith (1947), and Yevtushenko (1966), and that which will be available from the current CIIT study (CIIT 1979b) are deemed by the Agency to provide sufficient information to evaluate the chronic effects of chloromethane on the liver, kidney, bone marrow, and cardiovascular system. In the earlier studies the liver, kidneys, and bone marrow were affected, but at exposure levels higher than those that induced CNS effects. This

means that no-effect levels were in essence established for liver, kidney, and bone marrow toxicity. The no-effect levels varied with the frequency of exposure. For example, in rats exposed to 500 ppm, 6 hours per day, 6 days per week for nine months, no signs of liver, kidney, or bone marrow toxicity were detected (Dunn and Smith 1947), while exposure to less than 120 ppm was needed if the exposure frequency was 4 hours/day daily for 6 months (Yevtushenko 1966). Because no-effect levels have been determined for liver, kidney, and bone marrow toxicity under a series of test conditions and because the most sensitive indicator of toxicity appears to be the CNS, for which separate testing is being recommended, the EPA finds that no further chronic toxicity study to examine liver, kidney, and bone marrow toxicity is needed.

Effects on the cardiovascular system are associated with acute lethal concentrations of the chemical and not with non-lethal chronic exposure. As discussed in Section III.A., human and animal data are sufficient to evaluate the acute toxicity of chloromethane. Because of these two factors the EPA is not recommending further chronic studies to evaluate cardiovascular toxicity.

6. Epidemiology

The EPA has determined that at this time a suitable cohort for epidemiology studies cannot be identified and, therefore, is not requiring such studies. However, if information becomes available to the Agency through TSCA Section 8(a)(2)(F) leading

to the development of a suitable cohort, the Agency may reexamine this conclusion.

B. Exposure

Chloromethane was produced in the United States totally for domestic consumption at approximately 497 million pounds in 1979. Hydrochlorination of methanol is the process used for greater than 98 percent of production. It is used almost exclusively as an intermediate, primarily in the manufacture of silicones and tetramethyllead. Although chloromethane is present in the atmosphere in parts per trillion levels from natural sources, and in the parts per billion range from anthropogenic sources other than manufacturing, processing and use, the greatest risk of health effects is presented by exposure to chloromethane in local, high concentrations at the parts per million level found in occupational settings.

On the basis of chloromethane's almost exclusive use as an intermediate, reports prepared for NIOSH, and various reports of exposure found in the literature, the EPA staff concludes that the maximum potential for the possible risk associated with direct exposure to chloromethane exists during its manufacture, processing and use.

C. Material To Be Tested

The EPA is proposing that a grade of chloromethane of 99.97 percent or better be used as the test material in the required tests. Chloromethane of this purity is available commercially,

and is being used by CIIT in their studies. The primary reason for this proposal is that the EPA believes that the molecular species CH_3Cl, may pose an unreasonable risk and that, therefore, the most valuable information to evaluate the risks of exposure to chloromethane would come from testing the purest form of the compound available. The contaminants present in chloromethane produced by both of the industrial processes in use today, are either characterized or under deliberation for further testing under Section 4(a) of TSCA. General considerations for selection of the appropriate form of the substance for testing are discussed in the Legal and Policy generic document (Appendix 1 of Rule 1). Those manufacturers, processors or users of chloromethane may apply for a blanket exemption as dealt with in the Exemption Policy generic discussion (Appendix 3 of Rule 1).

I. Identity of Chloromethane

Chloromethane, CH_3Cl, (also known as methyl chloride) is a colorless, noncorrosive, liquefiable gas at room temperature and normal atmospheric pressure. Other physical properties of this chemical include: molecular weight, 50.49; boiling point, -23.7°C; melting point, -97.6°C; specific gravity, 0.92 at 20°C; solubility in water, 0.74 g/100 ml at 25°C (DeForest 1979); vapor pressure, 5 atm at 20°C; and an estimated logarithm of the

octanol/water partition coefficient (log P_{oct}) of 1.08 (Leo et al. 1971).[1]

Almost all of the chloromethane produced in this country (greater than 98 percent) is made by the hydrochlorination of methanol (Lowenheim and Moran 1975, CMR 1976). Ahlstrom and Steele (1979) state that two grades of chloromethane are produced, the technical and the refrigerant. The refrigerant grade must be very pure, to prevent attack by impurities on the refrigeration equipment, and generally contains less than 75 ppm water. Continental Oil Company produces chloromethane of at least 99.9 percent purity for the production of tetramethyllead, while Dow Corning, which uses chloromethane both as a direct contact refrigerant and in non-refrigerant uses, reports that the chloromethane it produces is greater than 99 percent pure (SRI 1979a,b). In response to a questionnaire, industry reported finding methanol, acetone, dimethyl ether, water, ethyl chloride, and hydrogen chloride as impurities in chloromethane (NSF 1975). Ahlstrom and Steele (1979) state that the known contaminants of a technical grade product are no more than 100 ppm H_2O, vinyl chloride, ethyl chloride, and residue, 50 ppm methanol and acetone, 20 ppm dimethyl ether and 10 ppm hydrogen chloride. It has also been reported that chloroform (trichloromethane) and carbon tetrachloride (tetrachloromethane) are obtained as coproducts in the production of chloromethane by

[1]With the exception of the solubility in water and the log of the octanol/water partition coefficient, all physical properties were obtained from recent editions of the Handbook of Chemistry and Physics (Weast 1978) and the Merck Index (Windholz 1976).

the hydrochlorination of methanol (SRI Undated, SRI 1979a), so that possible contamination by these products may also occur.

A small amount of chloromethane may be produced by direct chlorination of methane, in which case there is the potential for contamination with, in decreasing order, dichloromethane, chloroform, and carbon tetrachloride (Lowenheim and Moran 1975).

II. Exposure Aspects

A. General

In 1976 chloromethane was produced in the United States by 11 manufacturers at 15 sites (OTS 1979, SRI 1979a). In 1979, the Chemical Marketing Reporter (CMR) stated that U.S. production capacity is approximately 625 million pounds as produced by nine manufacturers at eleven sites (CMR 1979). The production volume in the United States averaged about 450 million pounds per year between 1970 and 1976, ranging from 544 million pounds in 1973 to 304 million pounds in 1975 (ITC 1970-1975). CMR reported that demand for chloromethane was 485 million pounds in 1978, 497 million pounds in 1979, and an estimated 550 million pounds in 1983, a growth rate of 2-3 percent per year through 1983, a result mainly of the growth potential of silicones (CMR 1979). The quantities of chloromethane that are either imported or exported are insignificant (EPA 1977).

Chloromethane is used almost exclusively as an intermediate. Approximately 50 percent of all chloromethane is consumed in the manufacture of silicones which are used for a wide variety of

products (CMR 1979). About 30 percent of chloromethane consumption is for the production of tetramethyllead, an antiknock compound used in gasoline formulations. This use is probably declining in the United States as a result of recent restrictions on the use of lead in gasoline, although tetramethyllead is being exported (CMR 1979).

Minor uses of chloromethane as a methylating agent in the production of methyl cellulose, as an intermediate in the production of quaternary amines, and as an intermediate in the production of certain pesticides account for about 4 percent each of total consumption. A variety of other intermediate uses such as in the production of Triptane©, an antiknock fuel additive, and methyl mercaptan, used to produce jet fuel additives, account for about 4 percent of total consumption.

The major nonintermediate use of chloromethane, which accounts for about 4 percent of consumption, is as a catalyst-solvent in the manufacture of butyl rubber. Minor nonintermediate uses of chloromethane are as a foam-blowing agent for extruded polystyrene foams, e.g., Styrofoam^{R} (EPA 1975, 1976, NAS 1978, SRI Undated) and as a direct contact refrigerant (SRI 1979b). At one time chloromethane was used widely as a refrigerant in both domestic and industrial refrigerators. Although there are some refrigeration devices using chloromethane still in operation today, this use has been almost completely replaced by other substances, notably the chlorofluorocarbons. Chloromethane is also used as an aerosol propellant combined with

dichloromethane, propane, and Freon 12 for various aerosol mixes (DeForest 1979).

B. Direct Exposure

Virtually all of the information on direct exposure to chloromethane involves occupational exposure. Because chloromethane is a gas at room temperature, the major route of human exposure to chloromethane is almost certainly inhalation. The 1972-74 National Occupational Hazard Survey (NOHS) indicates that an estimated 50,575 workers have the potential for exposure to chloromethane (NIOSH 1972-74).

The National Institute for Occupational Safety and Health (NIOSH) has sponsored studies in several plants that produce or use chloromethane, to evaluate the extent of worker exposure in various occupational settings. The exposure levels found in these studies, described below, were generally at or below the current threshold limit values (TLVs) for the time weighted average (TWA) of 100 ppm (210 mg/m^3) and the short-term exposure limit (STEL) of 125 ppm (260 mg/m^3) (ACGIH 1979). (The current TLV, and the data used to arrive at it are discussed in Section II.D.).

At the Dow Corning plant site in Midland, Michigan, chloromethane is used in the production of methyl chlorosilanes in three buildings, and is used as a direct contact refrigerant in three other buildings (SRI 1979b). In the first industrial hygiene survey conducted by SRI International (SRI) at the Dow Corning Corporation on September 27, 1977, (SRI 1979b) it was

determined that airborne chloromethane levels in the working environment ranged from less than 1 ppm to 51 ppm (as area samples). In March 1979 SRI conducted a personal monitoring survey of the operators at the Dow Corning plant site for full shift TWA exposure concentrations, and determined levels of chloromethane from below detection to 12.67 ppm on operators, and short-term levels of 0.62 ppm to 5.81 ppm on maintenance workers. Eight-hour TWA concentrations in four work areas were determined to range from below detection to 31.66 ppm. The highest levels were consistently found in chloromethane compressor areas. No samples were taken from areas in which chloromethane is used as a refrigerant. Although the chloromethane is stored, transferred, and reacted in relatively closed systems, chloromethane is nevertheless present in the working environment air. Furthermore, elevated short-term exposure levels of chloromethane can occur through a leak or when operators must collect quality-control samples. At least 38 employees work directly within areas of the plant that produce and use chloromethane. Additional workers with the potential for exposure include maintenance personnel, material handlers and laboratory personnel.

Dow Chemical uses chloromethane as a foam-blowing agent in its polystyrene (Styrofoam^R) foam process (NIOSH 1978). The Styrofoam^R production occurs in a closed system until the material comes through a die in the extruder and expands onto a conveyer assembly. Employees are exposed to chloromethane in the foam production area. Exposure is also known to occur when

chloromethane is liberated from the foam product while it is cooling and in storage, or when the residual chloromethane is released by certain operational procedures such as cutting, routing, drilling, and reaming of the finished product. Levels of 105 parts per trillion (ppt) (0.0001 ppm) and 355 ppt (0.00036 ppm) were found in two air samples collected from the foam storage warehouse (NIOSH 1978). The average eight-hour TWA exposure to chloromethane found in an SRI study ranged from 15 ppm to 54 ppm at various sites in the Styrofoam© plant, with the highest eight-hour TWA level being 101 ppm (NIOSH 1978). In another SRI study, average half-hour concentrations at sample points in Dow's fabrication plants ranged from 2-1500 ppm (SRI Undated). In 1969 Dow Chemical conducted a survey of nine in-plant chloromethane-containing manufacturing operations using continuous monitoring devices for four months for 54 job classifications. Time weighted average concentrations ranged from 5-78 ppm with an average 30 ppm concentration. Peak concentrations were as high as 440 ppm, but the duration of peak concentration exposure was not reported (SRI Undated).

DuPont Corporation produces chloromethane and uses it in the production of tetramethyllead (SRI 1978a). Tetramethyllead is produced in a closed system by the reaction of chloromethane with a sodium-lead alloy and aluminum chloride. Unreacted chloromethane is pumped to a recovery unit. The duration of exposure to chloromethane for employees in the production area may be up to eight hours per work shift. A concentration of 209 ppm was found in the tetramethyllead compressor room. In three operating

areas where chloromethane is used, short-term levels ranging from undetectable to 71 ppm of chloromethane were found. Chloromethane exposure levels (as TWA) were 6 ppm to 57 ppm in the chloromethane manufacturing facility, 2 ppm to 75 ppm in the tetramethyllead manufacturing facility, and 1 ppm to 34 ppm in the chloromethane recovery area.

Continental Oil Company (Conoco) produces chloromethane with potential exposures in the production area and in the tank-car loading operations (SRI 1978b). In an industrial hygiene survey done by SRI at the Conoco Chemicals facility in Westlake, Louisiana, on October 18-19, 1977, it was determined from sampling data that airborne chloromethane levels in the working environment ranged from 3 to 36 ppm (as area samples) (SRI 1979a). Simultaneous sampling by Conoco showed chloromethane concentrations ranging from less than 1 to 58 ppm. Personal sampling data accumulated by Conoco since 1975 in their quarterly sampling program showed six-hour TWA concentrations in the breathing zone ranging from 0 to 67 ppm for all chloromethane job classifications. (Fourteen employees were working in the chloromethane work areas at the time of the SRI survey.) SRI sampling data showed eight-hour TWA chloromethane concentrations determined from personal monitoring varying from less than 0.2 to 7.5 ppm. Average eight-hour TWA concentrations in 11 work areas ranged from 0.7 to 55.7 ppm. The highest concentrations were found in the compressor areas (SRI 1979a).

Chloromethane and methyl chlorosilane manufacturing facilities are located at a General Electric plant site (SRI 1978c).

Workers were reported to be exposed to chloromethane levels of 0.8 ppm to 75 ppm in the manufacturing facility, recovery unit, and compressor room from three to five hours each day.

Chloromethane is also used in the manufacturing process of four herbicides: paraquat (1,1'-dimethyl-4,4'-bipyridinium); DSMA (disodium methylarsonate); MSMA (monosodium methylarsonate); and cacodylic acid (dimethylarsinic acid) (Sittig 1977). Paraquat is made by the reaction of 4,4'-bipyridyl and chloromethane in water. MSMA and DSMA are final products after sodium arsenite is treated with gaseous chloromethane. This reaction takes place in a closed system; additional chloromethane is consumed in a side reaction with sodium hydroxide. In the production of cacodylic acid, chloromethane is added to the reaction chamber throughout the reaction, then the excess is bled off. No data were found on the occupational exposure to chloromethane in these four herbicide manufacturing processes, although the possibility of low-level, constant air concentrations or high-level, intermittent concentrations exists, as in other manufacturing processes using chloromethane. No information was found on possible chloromethane contamination of these pesticides.

All of the above direct exposure cases illustrate occupational exposure to chloromethane during its manufacturing, processing, and use. The EPA believes that occupational exposure to chloromethane presents the most substantial risk of exposure.

C. General Population Exposure[1]

Chloromethane appears to be the most abundant halocarbon present in the atmosphere (Lovelock 1975, Singh et al. 1977). SRI (1979c) have reported that chloromethane has an average background tropospheric concentration of 611 parts per trillion (ppt) (0.0006 ppm) in the northern hemisphere, and 615 ppt (0.0006 ppm) in the southern hemisphere. It is believed that this similarity indicates that the anthropogenic sources are relatively unimportant contributors to the atmosphere, rather than that extensive mixing occurs in the upper levels. Lower stratospheric levels are approximately 5 percent less (Cronn et al. 1977).

Chloromethane is decomposed when it reacts with hydroxyl radicals in the troposphere, with a small fraction reaching the upper stratosphere, where it is destroyed by photolysis (NAS 1976). The National Academy of Sciences (NAS 1976) estimated that the residence time of chloromethane in the atmosphere is about one year. More recently, SRI (1979c) estimated the residence time in the atmosphere to be 231 days.

The National Academy of Sciences (1976) reported an estimated total global emission rate (that is, both natural and anthropogenic emissions) of 14.7 billion pounds per year, based on an average global concentration of 750 ppt (0.00075 ppm). Two

[1]The level of detail in this subsection is unnecessary for the EPA's conclusions regarding the need to require health effects testing for chloromethane. However, the EPA is also evaluating the possible environmental effects of chloromethane (to be published later), and the General Population Exposure subsection is applicable to that assessment as well.

years later they estimated that the worldwide industrial emissions of chloromethane were 17.4 million pounds in 1973, only about 0.1 percent of the total emissions (NAS 1978). The estimated intentional and unintentional U.S. chloromethane release to the atmosphere from its production, transport, storage, use, and presence as an impurity in other products, amounted to 11.4 million pounds in 1973, approximately 2 percent of the annual U.S. production volume (NAS 1978). The EPA (1975) estimated that the United States accounted for approximately 60 percent of worldwide chloromethane production in 1973 and that approximately 10.5 million pounds of chloromethane was released from industrial activities in the United States in that year. Singh et al. (1979) estimate that 5-10 percent of annual U.S. production is emitted to the atmosphere. For example, Dow Corning vents escaping chloromethane from the manufacturing area through a stack to the outside air (SRI 1979b). However, industry responses to questionnaires indicated that the fraction of total annual production that escapes from the plant site to the atmosphere during manufacture of chloromethane is 0.0011-0.005 (NSF 1975). Assuming that the 2 percent release rate applies worldwide, the total release of chloromethane would not exceed 20 million pounds. However, using Singh et al.'s (1979) estimated 5-10 percent release rate, total release could be as high as over 50 million pounds annually. Either way it appears that industrial emissions of chloromethane are only a small fraction of the total of the more than 11-15 billion pounds estimated to be entering the atmosphere annually.

It is believed that the oceans constitute a major natural source of chloromethane. Singh et al. (1979) reported that the average surface concentration of chloromethane in the Pacific Ocean is 26.8 x 10^{-9} g/liter (26.8 ppt). It has been suggested that iodomethane, found ubiquitously in ocean water, reacts with chloride ion in the ocean surface water to form chloromethane, which then diffuses into the atmosphere (NAS 1976). Singh et al. (1979) have calculated that 6.6 billion pounds of chloromethane enters the atmosphere annually from the oceans.

It has also been suggested that burning vegetation is another important natural source of chloromethane. Palmer (1976) calculated that forest fires in the United States are responsible for about 252 million pounds per year of chloromethane released (average for 1972-1974). An additional 5.4 million pounds per year was calculated by Palmer to have been released from agricultural burning.

Another possible source of chloromethane is from photolytic decomposition of higher alkyl halides in the environment. The photolysis of gaseous chloroethane (a solvent) gives rise to chloromethane is one of the products (Cremieux and Herman 1974), which suggests that levels in the atmosphere may be less static than is implied by the relatively long residence time estimated by either the National Academy of Sciences (1976) or SRI (1979c).

Although it is clear from the above information that major sources of atmospheric chloromethane are natural, anthropogenic, i.e., resulting from human activities, sources may be responsible

for significantly elevated local concentrations. For example, Singh et al. (1979) reported that they found elevated urban concentrations of chloromethane in Lisbon [2.20 ppb (0.0022 ppm)] and near Los Angeles [average 1.50 ppb (0.0015 ppm); maximum 3.80 ppb (0.0038 ppm)]. They have suggested that automobile exhaust may be an important source of chloromethane. Palmer (1976) estimated that about 120 million pounds of chloromethane is released annually from building fires and 40 million pounds from the burning of polyvinyl chloride (PVC) in wastes (average for 1972-1974). The latter source was recognized by Palmer to be decreasing as the burning of such wastes was declining. The National Academy of Sciences (1978) estimated that tobacco smoking worldwide results in about 44 milion pounds of chloromethane entering the atmosphere annually. Based on average air intake of 23 m^3/day, and mean chloromethane concentrations over Los Angeles, Phoenix, and Oakland of 3.00 ppb (0.003 ppm), 2.39 ppb (0.0024 ppm), and 1.07 ppb (0.0011 ppm), respectively, the average human dose of chloromethane was calculated to be 140 ug/day, 109 ug/day, and 60 ug/day at the three sites respectively (SRI 1979c).

Elevated levels of chloromethane can occur in indoor air. Measurements of chloromethane in various contained atmospheres showed between 0.65 ppb (0.00065 ppm) and 8.00 ppb (0.008 ppm) by volume in various automobiles, 1.4 ppb (0.0014 ppm) in a restaurant, and over 20 ppb (0.02 ppm) in an apartment after a cigarette was smoked (Harsch 1977). Chloromethane was generally the predominant halomethane found in indoor air, and was

typically present at between two and ten times the ambient outdoor level (Harsch 1977, NAS 1978). It was suggested by the National Academy of Sciences (1978) that these elevated indoor levels may be due to cigarette smoking.

Chloromethane is found primarily in the air and ocean surface water, although it has also been qualitatively detected once in United States river water, three times in effluents from chemical plants, twice in effluents from sewage treatment plants, and eight times in drinking water (EPA 1979d), very possibly from the chlorination treatment of drinking water (EPA 1977, OWPS 1979). For the protection of human health from the toxic properties of chloromethane ingested through water and through contaminated aquatic organisms, the ambient water criterion level for chloromethane is 2 ug/l (OWPS 1979).

Although chloromethane is present in the atmosphere at a background parts per trillion level from natural sources (e.g., ocean waters) and at a parts per billion level in urban atmospheres from anthropogenic (e.g., cigarette smoke) sources, the EPA believes that the local, high concentrations of chloromethane in the parts per million levels found in occupational settings present the greatest risk of health effects resulting from exposure to chloromethane.

D. Existing Guidelines and Standards for Chloromethane

1. Threshold Limit Value

The TLV for chloromethane, 100 ppm, is based in part, upon the studies performed by Smith and von Oettingen (1947a,b) in

which the exposure of a number of different species of mammals to 300 ppm chloromethane for 64 weeks at 6 hours per day, 6 days per week, caused no observable detrimental effects to the animals. In addition, 9 unpublished in-plant surveys by Dow at their chloromethane manufacturing plants indicated that human illnesses were associated with exposures of 195 ppm and above, whereas exposures between 15 ppm and 195 ppm were not associated with reports of ill effects (ACGIH 1971).

The American Conference of Governmental Industrial Hygienists (ACGIH) is proposing to drop the present TLV to 50 ppm, on the basis of some of the literature discussed in later sections (see Section III) (ACGIH 1979).

2. Warning Label Required by Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Chloromethane is classified as an economic poison. Interpretation with respect to warning, caution, and antidote statements is required to appear on the label of technical chloromethane (USDA 1962).

3. Food Tolerance Requirement of Federal Food, Drug and Cosmetic Act

Chloromethane is regulated as a food additive permitted in food for human consumption as follows:

a. Its use as a propellant in pesticide formulations in an amount not to exceed 30 percent of the finished formulation is permitted.

b. It is restricted to use in food storage and processing areas where spray applications do not contact fatty foods.

c. To assure safe use of the additive, the label and labeling of the pesticide formulation containing the food additive shall conform to the label and labeling registered by the United States Department of Agriculture (FDA 1962).

4. Other

a. Chloromethane is one of the chlorinated hydrocarbons under consideraton for addition to the list of compounds for Toxic Effluent Standards (U.S. EPA Water Program Proposed Toxic Pollutant Effluent Standards) (EPA 1973).

b. It is listed on EPA Consent Decree Priority II List as one of the 129 Priority Pollutants (OWPS 1979).

III. Health Effects

A. Systemic Effects

Chloromethane exposure has been reported to result in a wide range of systemic toxicity following both acute and chronic exposure. Although effects on the liver, kidney, heart and hematopoietic system have been demonstrated in both humans and animals, the most sensitive organs seem to be the central nervous system (CNS). The available animal studies appear to be adequate for determining chronic toxicity in systems other than the CNS.

1. Human Studies

The EPA is not aware of any epidemiology study which evaluates the systemic effects of chloromethane on humans exposed chronically. However, there is a substantial case history literature of poisoning in humans, beginning with Gerbis' paper in 1914. Smith and von Oettingen (1947a) tallied the number of published chloromethane intoxication cases. By 1947 there had been 210, and 15 were fatal. The majority of poisonings before 1960 occurred from exposure to its use as a refrigerant (see e.g., Kegel et al. 1929, Schwarz 1926), while present day poisonings in this country appear to occur mainly in the rubber

and plastics industries (see e.g., Hansen et al. 1953, MacDonald 1964, Scharnweber et al. 1974).

Most of the case histories are believed to have involved acute exposures to levels of the chemical well in excess of the currently accepted TLV of 100 ppm (see e.g., Gerbis 1914, Kegel et al. 1929, Laskowski et al. 1976, Schwarz 1926) although idiosyncratic responses to low levels could conceivably account for some known instances of poisoning. In mild cases of acute poisoning, the toxic manifestations are primarily neurologic in character, as are those in chronic intoxications (see Section III.B.). However, gastrointestinal effects such as nausea, vomiting, and diarrhea are also prominent (see e.g., Mackie 1961, Sharp 1930, van Raalte and van Velzen 1945, Wiernikowski et al. 1974). Elevated body temperatures, pulse rate and heart rate are commonly reported (see e.g., Hansen et al. 1953, Kegel et al. 1929, Laskowski et al. 1976), while depressed blood pressure (see e.g., McNally 1946, Suntych 1956, Trubecka and Brzeski 1968, Weinstein 1930), and abnormal EKG readings (see e.g., Gaultier et al. 1965, Gummert 1961, Noro and Pettersson 1960, Walter and Weiss 1951) also indicate cardiovascular involvement.

The other organs or systems primarily influenced by chloromethane are the liver, kidney, and blood. Hepatic damage occurs in acute cases (see e.g., Saita 1959, Spevak et al. 1976, Weinstein 1930), and in long-term exposures (see e.g., Del Zotti and Gillardi 1954, Mackie 1961, Wood 1951), while kidney damage manifests itself as renal insufficiency and anuria in the more severe cases (see e.g., Borghetti and Gobbato 1969, Hayhurst and

Greenburg 1929, Kegel et al. 1929, Suntych 1956), and proteinuria which is representative of less severe damage (see e.g., Birch 1935, Mackie 1961, McNally 1946).

The hematologic picture is not as clear. Although some investigators have seen anemia (see e.g., Hayhurst and Greenburg 1929, Kegel et al. 1929, Mackie 1961, Milkov et al. 1965) and others leukocytosis (see e.g., McNally 1946, Noro and Pettersson 1960, Suntych 1956, Wiernikowski 1974); in some instances, even following severe poisonings, the blood cell counts remain within normal levels (see e.g., MacDonald 1964, Spevak 1976, van Raalte and van Velzen 1945, Weinstein 1937).

Although most exposures to chloromethane are assumed to be by inhalation, the lung apparently is relatively insensitive to the chemical.

2. Animal Studies

There have been few studies on the effect of repeated exposure to chloromethane in animals. Details of four of the most relevant of these studies follow.

An experiment was undertaken by White and Somers (1931) for the purpose of determining the minimal concentration of chloromethane which would cause death in average-sized guinea pigs when the exposure via inhalation covered a 72 hour period. After the exposure period, the animals were observed for an additional thirty days. Each of three groups of animals (18 animals per group) was exposed to an average concentration of 49, 77, or 140 ppm. In the group exposed to an average of 49 ppm, none of the

animals died within the 30 day observation period; in the group exposed to an average of 77 ppm, 50 percent of the guinea pigs died; and in the group exposed to an average of 140 ppm, all of the animals died within a few days after exposure. The pathologic changes in the guinea pigs dying from the effects of chloromethane indicated widespread systemic poisoning characterized mainly by severe circulatory disturbances and congestion of the lungs and meninges.

Smith, von Oettingen, and Dunn conducted an extensive study of the acute and chronic toxicity of chloromethane (Smith and von Oettingen 1947a,b, Smith 1947, Dunn and Smith 1947). They studied the mortality, symptomatology, effects on hematopoietic and biochemical parameters, and the histopathologic changes resulting from exposure to chloromethane. In these studies, the chemical was administered to 10 species of animals via inhalation 6 hours/day, 6 days/week for up to 64 weeks, at concentrations of 300-4000 ppm.

Table 1 summarizes the mortality data at 500 and 2000 ppm in terms of the number of days from first exposure to death of 50 percent of the experimental animals (LT50) (Smith and von Oettingen 1947a). The most sensitive species at the 500 ppm concentration was the dog, the least sensitive was the rat. No apparent effect was noted in guinea pigs, mice, dogs, monkeys, rabbits, and rats exposed to 300 ppm, 6 hours/day, 6 days/week for 64 weeks. The other four species were not exposed to this concentration.

TABLE 1

Mortality of Animals Exposed to Chloromethane
6 Hours/Day, 6 Days/Week
LT50 (Days)

Species	500 ppm	2000 ppm
Guinea pig	71	3
Mouse	143	3
Goat	NS[a]	3
Dog	23	4
Monkey	110	10
Rat	NE[b]	15
Rabbit	192	23
Cat	NS	27
Chicken	NS	38
Frog	NE	NE

[a]NS--not studied
[b]NE--not lethal

Smith and von Oettingen (1947a) also found that several factors influenced survival time within a species. These factors included exposure frequency, age, and certain dietary constituents. As shown in Table 2, the interval between exposures (i.e., exposure-free period) greatly influenced the mortality rate. Allowing exposure-free periods apparently decreases the cumulative effects of chloromethane. The work of White and Somers (1931) also supports this thesis. In their study, the LD_{50} for guinea pigs exposed to chloromethane continuously for 72 hours was only 77 ppm, indicating that uninterrupted contact is much more lethal.

TABLE 2

Mortality of Animals Exposed to Chloromethane for Different Exposure Intervals

Species	Concentration (ppm)	Exposure Time (hours/day)	Frequency (days/week)	LT50 (days)
Mouse	1,000	6	6	5
	2,000	3	6	131
	2,000	6	6	3
Guinea	2,000	6	6	3
Pig	2,000	6	3[a]	201

[a]Three alternate days a week

Variation in age also influenced mortality. Younger animals appeared to be more resistant than older animals. For example, when adult and weanling rats were exposed to 2000 ppm, 6 hours/day, 6 days/week, the LT50 for the adult animals was 15 days, while that for the weanlings was 27 days.

Supplementing the diet of guinea pigs exposed to 1000 ppm with ascorbic acid, or the diet of rats exposed to 2000 ppm with thiamine hydrochloride, nicotinic acid, or calcium pantothenate did not increase resistance to the lethal effects of chloromethane. However, increasing dietary casein by 20 to 35 percent or supplementing moderate to low casein diets with cystine or methionine led to an increase in the time before 50 percent of the rats died.

No differences in LT50 could be attributed to differences in sex.

Smith and von Oettingen (1947b) also studied the symptomatology of the animals poisoned by chloromethane. Commonly seen were anorexia, discharge of fluid from the respiratory tract, hyperactive reflexes, disturbances in ability to correct position, and extreme spasticity. The neurologic behavior of monkeys (tonic-clonic convulsions and periods of unconsciousness), was different from that of dogs (sustained tonic spasms without remission). However, both types of neurotoxicity have been reported in humans (see Section III.B.).

Development of symptoms varied with concentration and frequency of exposure. Symptoms were delayed or gradual with low concentrations or with high concentrations separated by longer exposure-free intervals. Young animals responded with a slow development of symptoms at some concentrations where older animals developed symptoms acutely. Although animals generally recovered from acute symptoms if exposure was discontinued when symptoms first appeared, symptoms acquired over a long period of time were sometimes irreversible.

Smith (1947) reported hematology and biochemical results on certain of the animals studied by Smith and von Oettingen (1947a,b). No hematologic or biochemical test was purported to be useful in the diagnosis of chloromethane poisoning in the species studied. No evidence of liver dysfunction, of renal failure, or of a primary effect upon the formed elements of the blood were detected without severe neuromuscular disturbances having preceded the detected hematologic or biochemical

changes. These data indicate the CNS to be the system most sensitive to chloromethane toxicity.

Histopathologic examination on the same group of experimental animals was reported by Dunn and Smith (1947). Morphologic changes that appeared to be a direct result of inhalation of chloromethane 6 hours/day, 6 days/week, were variable degrees of necrosis of the convoluted tubules of the kidney in mice and rats (2,000 ppm) and fatty metamorphosis of the liver in the smaller species. Pulmonary edema appeared to be a direct result of the irritation due to inhalation of chloromethane. No morphologic changes were found in the brains of those dogs and monkeys examined which showed severe neuromuscular disturbances. No morphologic changes were observed in the rats exposed to 500 or 1000 ppm, 6 hours/day, 6 days/week for nine months. However, the tissues examined were not specified. Guinea pigs surviving 9 months of exposure at this level also demonstrated no histopathologic changes, although guinea pigs were the second most sensitive species (LT50) at 500 ppm while rats showed no lethality at 500 or 1000 ppm (Smith and von Oettingen 1947a).

The major limitations of the four studies done by Smith, von Oettingen, and Dunn are:

(1) small numbers of animals were used in certain test groups (e.g., two goats at 2000 ppm, four rabbits at 1000 ppm, two monkeys at 500 ppm);

(2) histopathologic examinations were not reported on animals exposed to 300 ppm for 64 weeks for species which showed effects at 500 ppm; and

(3) no indication of the animals or tissues routinely examined was given.

Therefore, while the studies indicate at what levels major effects of concern might appear, they are inadequate for determining a no-effect level.

Yevtushenko (1966) studied the chronic effects of chloromethane on rats (10 animals/group) and rabbits (4 animals/group), exposed to 40 or 240 mg/m^3 (i.e., approximately 20 and 120 ppm, respectively), 4 hours/day, daily for 6 months. In both groups of rats hematologic examination revealed consistent decreases in erythrocyte number. In both rats and rabbits exposed to 240 mg/m^3, excretory function of the liver was disturbed while no effect was observed in the animals exposed to 40 mg/m^3. In rats of both groups, kidneys functioned normally, but microscopic examination of the blood-forming organs indicated depletion of lymphoid elements and proliferation of the reticular base of the organs examined (spleen, lymph nodes). Changes in parenchymal tissues were unpronounced. The rabbits were also used to observe effects on the eyes. These were observed in both exposure groups and included discoloration of the optic disc and histopathologic disturbances of the retina and optic nerve. The most significant changes occurred in the CNS (see Section III.B.2.b.).

CIIT (1979a) sponsored a 90-day inhalation study using rats and mice. Groups of animals (ten animals/sex/dose) were exposed to 375, 750, or 1500 ppm of chloromethane 6 hours/day, 5 days/week for 13 weeks. The purpose of this study was to select appropriate exposure levels for a subsequent chronic toxicity study. All three groups of treated male rats showed a significant decrement in body weight compared to controls, while female rats treated with 750 and 1500 ppm also showed significant decrements in body weight compared to controls. There was a significant difference in final body weight between treated and control mice at only the 1500 ppm dose level in females, however. Weight loss, or a lag in weight gain is accepted as a sensitive and easily measurable method for observing toxicity. The top dose level in a combined chronic-oncogenicity test should be the maximally-tolerated dose, (MTD) and is defined to be the highest dose that causes no more than 10 percent weight decrement and is predicted to produce no lessons (other than those related to a neoplastic response) (EPA 1979). It is more difficult to decide what is the proper MTD in a species in which in the highest dose used caused little or no toxicity in preliminary testing.

The group of male mice treated with 1500 ppm had significantly higher serum glutamic-pyruvic transaminase (SGPT) activity than controls. However, this activity was increased in only two of the mice. One was found to have a liver infarction while the other had severe hepatic changes. One of the major problems with this study was the fact that although

7/10 male mice at the high dose died of trauma, 6 males are listed as the total on which final studies were done, without any indication as to dates of death, or which animals were showing which effects. Other clinical and hemotologic parameters measured were reported to be within the normal clinical range. The major histopathologic finding was cytoplasmic vacuolar changes of the hepatocytes in mice. Sixty-four percent (9/14) of the mice treated with 1500 ppm exhibited the effect, while 37 percent (7/19) of controls exhibited the effect. The effect was highest in females and was more pronounced in the treated group than in the control group. One female not in the high dose group had a massive liver infarction. Thirteen of the 60 treated mice had eye lesions, but it was concluded that this effect was not compound related. However, deficiencies in the design and conduct of this study, have lead the EPA to decide that the findings from it cannot be used as the sole determinant of the chronic toxicity of chloromethane (see Section IV).

Several major conclusions about the chronic toxicity of chloromethane can be drawn from the human studies and from the animal studies of White and Somers (1931), Smith and von Oettingen (1947a, b), Smith (1947), Dunn and Smith (1947), and Yevtushenko (1966). These conclusions are:

(1) chloromethane is toxic to a variety of species including humans;

(2) the major systems affected include the CNS, liver, kidney, blood forming elements, and ocular tissue;

(3) the most sensitive system affected in humans and animals appears to be the CNS; and

(4) the level of toxicity is not only affected by the exposure concentration but also by the length of the exposure-free period and the amount of cystine or methionine in the diet.

B. Neurotoxicity

Chloromethane is a non-specific central nervous system (CNS) depressant. There are human case reports, several animal studies, and controlled human laboratory studies that document its acute and chronic neurotoxicity. Chloromethane intoxication produces neurological signs, mood changes, and cognitive and intellectual deficits, as well as other symptoms. Neurological signs include ataxia, tremor, motor reflex changes, and signs of cranial nerve involvement such as blurred vision, weakened convergence, mydriasis, and vertigo. Mood changes such as apathy, irritability, euphoria in earlier stages of acute exposure and/or depression in later stages also occur. Cognitive deficits relate to difficulties in concentration and memory loss. More severe CNS alterations also occur in acute poisoning. Convulsions of both the tonic-clonic type (Hartman et al. 1955, McNally 1946)

and that characterized by sustained tonic contractures (Kegel el al. 1929, Schwarz 1926) have been seen. Other major symptoms are headache, fatigue, and sleep disturbances (Table 3). The onset of these signs and symptoms may be delayed by several hours following exposure and can persist indefinitely (see e.g., Gudmundsson 1977, Walter and Weiss 1951). While the information available is insufficient to completely characterize chloromethane for acute effects, the Agency deems it unnecessary at this time to require further testing for systemic effects resulting from acute exposure. However, chronic neurotoxicity, with a potential for permanent effects, is definitely indicated by the evidence as a risk to human health that is impossible to assess at this time without additional data, acquired by testing.

1. Acute and Sub-Acute Neurobehavioral Effects

a. Human Reports and Studies

There have been numerous human case reports of acute intoxication (see e.g., Noro and Pettersson 1960, Spevak et al. 1976, Thordarson et al. 1965, Wiernikowski et al. 1974). The first column of Table 3 shows the frequency and nature of reported signs and symptoms. These studies generally lack any quantitative estimates of levels or duration, which generally makes it difficult to use the information for hazard assessment purposes, or the establishment of a no-effect level.

While victims of acute exposure generally show complete and rapid recovery, very long lasting changes have also been reported. Gudmundsson (1977) did a follow-up study 13 years

Table 3. Neurologic Symptoms Seen in Man

	Acute							Acute & Chronic						Chronic	
References / Clinical Symptoms	Kegel et al. (1929) (7)[a]	Weinstein (1937) (2)	van Raalte & van Velzen (1945) (1)	McNally (1946) (8)	Noro and Pettersson (1960) (5)	Wiernikowsky et al. (1974) (3)	Spevak et al. (1976) (4)	Baker (1927) (21)	Sharp (1930) (2)	Hansen et al. (1953) (15)	Mackie (1961) (1)	MacDonald (1964) (8)	Scharnweber et al. (1974) (6)	Anon. (1945) (1)	Langauer-Lewowicka et al. (1974) (25)
visual disturbances	5[b]		1	2	+[c]	+		16		6	1	5	3		2
diplopia	1				+		2	4					2		3
ptosis	2							21							+
mydriasis	6			2			4								
anisocoria				2			4								5
nystagmus	1						4								
weakened convergence							2								3
strabismus	1						2								
photophobia							4								
dysphagia								3		2			1		
hiccup	3				+			1		2					
paresis of facial nerve			1				4								3
twitching muscles	3		1				4							1	
tremors	2		1	1		+	4	5	1				1		3
pyramidal signs						+	4								17

Table 3. Neurologic Symptoms Seen in Man

	Acute							Acute & Chronic						Chronic	
References / Clinical Symptoms	Kegel et al. (1929) (7)[a]	Weinstein (1937) (2)	van Raalte & van Velzen (1945) (1)	McNally (1946) (8)	Noro and Pettersson (1960) (5)	Wiernikowsky et al. (1974) (3)	Spevak et al. (1976) (4)	Baker (1927) (21)	Sharp (1930) (2)	Hansen et al. (1953) (15)	Mackie (1961) (1)	MacDonald (1964) (8)	Schannweber et al. (1974) (6)	Anon. (1945) (1)	Langauer-Lewowicka et al. (1974) (25)
visual disturbances	5[b]		1	2	+[c]	+		16		6	1	5	3		2
diplopia	1				+		2	4					2		3
ptosis	2							21							+
mydriasis	6			2			4								
anisocoria				2			4								5
nystagmus	1						4								
weakened convergence							2								3
strabismus	1						2								
photophobia							4								
dysphagia								3		2			1		
hiccup	3				+			1		2					
paresis of facial nerve			1				4								3
twitching muscles	3		1				4							1	
tremors	2		1	1		+	4	5	1				1		3
pyramidal signs						+	4								17

Table 3. Continued

References	Acute							Acute & Chronic						Chronic	
Clinical Symptoms	Kegel et al. (1929) (7)	Weinstein (1937) (2)	van Raalte & van Velzen (1945) (1)	McNally (1946) (8)	Noro and Pettersson (1960) (5)	Wiernikowsky et al. (1974) (3)	Spevak et al. (1976) (4)	Baker (1927) (21)	Sharp (1930) (2)	Bansen et al. (1953) (15)	Mackie (1961) (1)	MacDonald (1964) (8)	Scharnweber et al. (1974) (6)	Anon. (1945) (1)	Langauer-Lewowicka et al. (1974) (25)
Ataxia	2		1							8			1		
Rombergism			1				4								
Vertigo	1	2						21				1	1		+
Staggers			1					21		2		6	3		
Loss of balance													1		+
Dizziness	1		1			+			1	12		8	2	1	+
Adiadochokinesis							1								
Paresthesia			1												+
Sensitivity							3								+
Weakness in limbs				2	+								1		3
Generalized weakness	4		1	1	+					9	1	3		1	+
Apathy							1				1				
Tiredness, sleepiness	5		1				4	21		12		1	1		+
Euphoria							2					1			
Depression			1									4			

Table 3 Continued

References / Clinical Symptoms	Kegel et al. (1929) (7)	Weinstein (1937) (2)	van Raalte & van Velzen (1945) (1)	McNally (1946) (8)	Noro and Pettersson (1960) (5)	Wiernikowsky et al. (1974) (3)	Spevak et al. (1976) (4)	Baker (1927) (21)	Sharp (1930) (2)	Hansen et al. (1953) (15)	Mackie (1961) (1)	MacDonald (1964) (8)	Scharnweber et al. (1974) (6)	Anon. (1945) (1)	Langauer-Lewowicka et al. (1974) (25)
confusion	2				+					1		4	2		
nervous, worried	1									1		2	3		
irritable												2			
loss of memory	1					+				1		1	3	1	+
loss of libido											1	1			
psychosis															2
insomnia	1		1					15							
anorexia	1		1					21		1	1				
nausea	5	2	1	1	+			11	2	5	1	6		1	
speech disturbance	2		1			+	4			3		2	4		
headaches	2				+	+				1	1	7	1		+
hearing difficulties						+									
difficulty in concentration	1					+				1			1		
heavy head	1											2			
convulsions	2			1											

a) The number in parentheses following the year of the reference is the number of persons exhibiting chloromethane intoxication in the paper.
b) The number of patients exhibiting this particular sign.
c) If the number of persons exhibiting the sign was not mentioned, but the sign was reported as occurring, a (+) is indicated.

after 15 people were exposed to chloromethane from a leaking refrigerator on a fishing boat (Thordarson et al. 1965). One patient died within 24 hours of the incident, two suffered severe depression and committed suicide within 2 years of the incident, and one man who had been seriously disabled, died ten years later of a coronary occlusion. Ten of the eleven survivors were examined. Nine patients reported a reduced tolerance to alcohol and six had chronic fatigue and depression. Five patients showed neurological signs: 3 with tremor, 2 with paralysis of accommodation, and 2 with peripheral neuropathies complicated, however, by a history of alcohol abuse. Hartman et al. (1955) reported that 1.5 years following a severe acute exposure, a woman still displayed intention tremor, headaches, insomnia, and "nervousness". Few of the other studies in the literature have reported any long term follow-up.

There have been 2 recent laboratory studies of acute exposure in humans. Putz et al. (1979) reported behavioral performance deficits in a complex visual vigilance task during and after 3 hours of exposure to 200 ppm, but no effects at 100 ppm. Stewart et al. (1977) exposed 4 humans to 100 ppm for 5 days for 7.5 hours a day. Analysis by the authors revealed no impairment on a battery of neurologic and behavioral tests, including 2 timing tasks, one with no cues and one with auditory cues. But their analysis of variance revealed a significant npairment in a timing task that relied on visual cues. Although › investigators concluded that there was no cognitive irment of timing behavior, the EPA believes that the

demonstration of a visual system-related decrement in such a controlled study seems significant when considered in light of Putz's visual task deficits after 3 hours at 200 ppm.

b. Animal Studies

Yevtushenko (1966) reported that the four hour LC_{50} (lethal concentration in 50 percent of the animals) for rats was roughly 11,000 ppm. Depression of motor activity occurred, as well as widespread edema and vascular congestion of the brain and other organs. A four hour exposure to 114 ppm was reported to produce a behavioral deficit, namely, an increase in the time required to develop a conditioned reflex.

2. Subchronic and Chronic Neurobehavioral Effects

Signs and symptoms of chronic toxicity, based on case reports, do not differ qualitatively from those described above (see introduction to III.B.).

a. Human Reports and Studies

Based on a study of refrigerator workers, Klimkova-Deutschova (1957) suggested that fatigue, headache, sleep disturbances, and difficulty in concentration are among the earliest symptoms of chronic intoxication, and that cerebellar neurological signs predominate early while extrapyramidal signs are more frequent later on. In addition, onset of toxicity was insidious and once signs and symptoms appeared they were sometimes permanent. In many reports it appears that signs and

symptoms were reported in workers exposed both chronically at low levels and acutely at much higher levels from accidental spills or leaks (see e.g., Baker 1927, MacDonald 1964, Scharnweber et al. 1974; see also the second column of Table 3). This makes these studies difficult to evaluate in relation to separating chronic from acute effects. As in acute case reports, quantitative exposure data or correlation with employment duration are generally absent for both mixed and chronic exposure studies.

Belova and Yevtushenko (1967) performed detailed examinations of the visual system of chronically exposed workers. In those exposed for 2 to 3 years, roughly one-third showed a decline in corneal sensitivity and in some there was slight discoloration of the optic disc. Two-thirds of those exposed for 5 to 8 years showed a decline in corneal sensitivity. In addition, half of the workers displayed a complex group of visual changes.

Repko et al. (1977) performed a behavioral, neurological, and psychological study of chronically exposed workers (1-311 months, mean=84 months) in comparison with a control group. The exposed cases consisted of 171 "physically normal" paid volunteers from eight different plants at seven locations in six states (11 female/160 male, 10 black or minority/161 white). The controls (comparisons), who were matched (attempted) by sex, age, and race to the cases, consisted of 49 workers who were not known to be exposed to chloromethane or other neurotoxicants (3 female/46 male, 3 black or minority/46 white). Regardless of matching, the differences in mean age and level of education

and increases in resting tremor, but could only establish a suggestive relationship between these effects and airborne chloromethane levels.

The data analysis performed by Repko et al. to determine this relationship exemplifies the limitations of the study. Overall, the statistical comparisons are not sensitive to cumulative exposure simply because this information is not contained within the data. However, an attempt was made to establish a relationship between duration of chloromethane exposure (months employed) and physiologic effects of exposure. Duration of exposure was compared with measurements of breath chloromethane, urine pH, hematocrit, and ambient air concentrations. A statistically significant positive correlation was found between breath and air levels, and a negative correlation was found between air concentration and hematocrit levels. In subsequent analyses, correlations between neurologic effects and these physiologic variables were investigated. For the behavioral data, means from cases and controls were compared. Such a technique does not distinguish levels of exposure among cases. Behavioral data was investigated via scatter plots of ambient air concentration versus factors related to behavioral tasks. This comparison investigates the responses to various doses measured in the workplace. Repko et al. (1977) concluded that performance levels were reduced among workers exposed to chloromethane. Due to the aforementioned limitations of the study design, this reduction is only suggested rather than conclusively supported. No measurements of performance previous

Table 4. Neurologic Signs Occurring Following Continued Exposures

Air Concentration in ppm	Species	Effects
500	Mice	Convulsive activity occasionally occurred during the first week. After a week or more of exposure, mice developed a syndrome which began with a clamping of the hind legs to the body when the mouse was held up by the tail. This syndrome worsened with continuing exposure. Mice surviving 15 weeks of exposures retained the clamping response 6 months following termination of exposure.
	Dogs	Three of four dogs exhibited spasticity and staggering by the end of the second week. Two days later two were no longer able to stand and the third was shaking violently. Two died within the next 8 days and the third showed maximum extensor rigidity and opisthotonus when it was held off the floor. The fourth animal developed a slight ataxia after 4 weeks, lack of neuromuscular fine control following 2 months exposures, prominent tremors, ataxic gait and abducted hind legs when standing at 6 months. Tendon reflexes were hyperactive and spasticity increased when the dog was lifted off the floor. Exposures were discontinued after 29 weeks, and during the following 17 weeks there was no notable amelioration of the neuromuscular symptoms.
1,000	Guinea Pigs	Loss of righting reflex; hind leg displacement reflex retarded; later development of convulsions and opisthotonus within the first week. Following exposure to 3 weeks of chloromethane, neuromuscular signs first appeared and progressed until the guinea pig was unable to walk. At 12 weeks a regular flicking of the ears and a fine tremor was noticeable, but running a pencil along the mesh of the cage would initiate a convulsive episode. At 14 months, although many neuromuscular effects had disappeared, it still could not right itself.
	Dogs	Generalized tonic spasm with powerful opisthotonus and risus sardonicus; hyperactive reflexes and coarse tremors accompanied spasticity occasionally in the first week of exposure.

Concentration in ppm	Species	Effects
	Puppies	First symptoms appeared in second month of exposure when alternate gait was replaced with gamboling gait with frequent tumbling. In the third month the more severely affected pup showed tremors, and after 11 weeks intermittent convulsive seizures, attacks of hiccups, audible grinding of the jaws and risus sardonicus, and sustained contraction of tongue and jaw muscles. Exposures were discontinued at 12 weeks, and the general condition of the more severely affected animal improved for about one month, but after the fourth month, it grew worse and was sacrificed three months later. The other pup was observed for 10 1/2 months and the general condition was excellent, though the pup could not stand without sagging or swaying of the posterior trunk and legs and there was a tendency for the hind legs to remain displaced posteriorly.
	Rabbits	After several weeks exposure to methyl chloride rabbits were first unable to bring the hind legs to the normal position for hopping, and later the hind legs gradually became permanently adducted.
2,000	Chickens	After three weeks the legs became weak and abducted and the chickens unable to walk. Debility and paralysis increased until the entire body except head and neck were paralyzed and cold to touch.
	Cats	After a week, cats became weak, ataxic, lost righting reflex. Symptoms progressed until cats unable to walk and had frequent extensor spasm. Hyperactive reflexes.
	Rats	Rats on stock diet occasionally showed opisthotonus. On semi-synthetic diet, in which survival times were prolonged, the clamping syndrome seen in mice appeared after about 5 weeks of exposure, culminating in paralysis of the hind legs several weeks later. Residual abnormalities observed after 4 months in one rat.
	Goat	Following removal from the exposure chamber after the fourth exposure, the legs became rigidly extended and 20 minutes later spastic activity became general.
	Monkeys	Ataxia; poor hand to mouth coordination; one monkey developed convulsions followed by unconsciousness within the first week of exposure.

to exposure (pre-employment) were obtained. Therefore, there is no certain means of knowing whether workers' performance levels were affected by exposure.

b. Animal Studies

As noted above, the major study of chronic toxicity in animals (rats, mice, guinea pigs, rabbits, dogs, monkeys, others) was performed by Smith and von Oettingen (1947a,b) (see also Section III.A.2.). Neurological effects seen in the 500, 1000 and 2000 ppm groups exposed for 6 hours/day, 6 days/week are summarized in Table 4. In general, they found that 300 ppm for 64 weeks "had no apparent effect on any species tested", but that 500 ppm produced serious toxicity in most species, with particularly pronounced neurological signs in dogs and monkeys.

A Russian study (Yevtushenko 1966), which the author cited as one basis for the 1965 Soviet TLV of 2.5 ppm, reported behavioral and pathologic effects in rats and rabbits exposed for 4 hours/day to either 120 ppm or 20 ppm for 6 months. Development time of a food conditioned reflex to a bell increased in both groups of exposed rats compared to controls, while the natural conditioned reflex to the sight and smell of food was significantly delayed in rats exposed to 240 mg/m^3. Microscopic examination established that the brain was significantly affected in both groups of rats with vacuolization of protoplasm being the predominant kind of change noted in nerve cells.

Belova and Yevtushenko (1967) performed visual pathologic examination of the rabbits exposed in the same study. In the

initial weeks of the experiment, slight hyperemia of the conjunctiva and the appearance of a small amount of discharge from the eyes were observed. In animals of both groups (20 and 120 ppm) the optic disc was pale or grayish and frequently had diffused edges. Edema of the optic disc was noted in rabbits exposed to the higher level. Blood vessels of the eye were of uneven caliber, arterial vessels were primarily constricted, and small hemorrhages were noted in the retina of some animals. Histologic examination of the retina and optic nerve indicated morphologic changes, as well as increased vascularization, plasmorrhagia and hemorrhaging. No information on the optic system of the rats exposed at the same dose was given.

In the 90-day inhalation study of chloromethane sponsored by CIIT (see Section IV), rats exposed to 375, 750, or 1500 ppm exhibited no gross pathologic alterations of the eye. However, mice exposed to the 375 and 750 ppm dose had a high incidence of eye lesions that began as a mucopurulent conjunctivitis and progressed until some animals' eyes were totally destroyed (CIIT 1979a).

The following conclusions on the neurotoxicity of chloromethane can be made:

(1) adult dogs and monkeys appear to be the species most sensitive to the neurologic motor effects of chloromethane; and

(2) humans appear to show both the type of neurologic and behavioral toxicity expressed in monkeys and that expressed in dogs.

C. Mutagenicity

Chloromethane has been reported to possess mutagenic activity in bacterial systems that detect gene mutations (Andrews et al. 1976, DuPont 1978, Simmon et al. 1978), and to cause chromosomal aberrations in higher plants (Smith and Lotfy 1954). However, the evidence for chloromethane mutagenicity from this series of experiments is insufficient to permit a mutagenicity hazard assessment for chloromethane. To perform such a hazard estimation for humans, scientists must first demonstrate that a substance and/or its metabolite(s) does or does not cause heritable gene or chromosomal mutations (the two classes of mutagenic damage which have been shown to be responsible for a portion of human genetic disease) in a higher system, and whether or not the mutagenically active form can reach the genetically significant target molecules in mammalian germinal tissue.

1. Gene Mutation

A review of the data available shows that chloromethane is a direct-acting mutagen. This means that chloromethane does not have to be metabolized by mammalian enzymes to an active form. In bacterial systems capable of detecting gene mutations, chloromethane produces a strong, positive, reproducible dose-response curve of chemically induced mutations in *Salmonella*

typhimurium strains TA 1535 and TA 100. These strains normally cannot synthesize the amino acid histidine and this must be added to the nutrient medium to support their growth. When the proper mutation occurs in the specific portion of deoxyribonucleic acid (DNA) of these organisms that regulates this effect, they are able to synthesize histidine and are then capable of growth in histidine-free medium. The bacterial strains (TA 1535 and TA 100) which demonstrate chloromethane mutagenicity are mutated by agents which cause changes in a specific guanine-cytosine base pair as well as others in the DNA molecule. Agents that cause such changes are called base pair mutagens. S. typhimurium TA 1535 and TA 100 are the same basic strain; TA 100 is TA 1535 with the addition of a resistance factor, pKM 101, which confers resistance to the antibiotic ampicillin and, at the same time, increases the sensitivity to mutagenic agents.

After exposure to chloromethane, both with and without metabolic activation, increased numbers of bacteria of strains TA 1535 and TA 100 were capable of growth in histidine-free medium. On a quantitative basis, increasing concentrations of chloromethane caused the mutation of greater numbers of bacteria. For example, in a population of approximately 1×10^8 strain TA 100 bacteria, there will ordinarily be approximately 100 bacteria capable of growth in histidine-free medium. Exposure to 2.5 percent chloromethane increased this number to approximately 400 in 1×10^8; exposure to 20 percent chloromethane increased the number to approximately 1,100 in a total population of 1×10^8 bacteria (Simmon 1978). Similar results

were reported by Andrews et al. (1976), and DuPont (1978). In this test system, therefore, chloromethane is a base pair mutagen which causes an alteration in at least one guanine-cytosine portion of the DNA molecule.

DuPont (1978), however, tested chloromethane in two strains of S. typhimurium that detect frameshift mutagens, TA 98 and TA 1537, and reported it to be inactive. It is not uncommon for a chemical which is a positive base pair mutagen to be inactive in a frameshift strain and vice versa. This is one reason that a good Ames test will include strains of both types of S. typhimurium. However, the EPA believes that positive results in only one strain are considered adequate to determine that a chemical may pose a risk of human mutagenicity and should be tested further. The tests the EPA proposes for assessing hazard (see Summary B. 2. a) do not specifically measure base pair or frameshift mutations, but are valid for both types.

Given the universality of the structure of DNA, it is reasonable to assume that chloromethane may also cause base pair alterations in the DNA of higher organisms, including man.

The Diamond Shamrock Corporation (1978a) has submitted a series of test results in which chloromethane is reported to be non-mutagenic for S. typhimurium strains TA 1535 and TA 100 and Escherichia coli ATCC 23221 and ATCC 23233 and inactive in a host-mediated assay in mice with strain TA 100 as the tester strain. However, the significance of these negative test results is questionable because of the experimental techniques reported.

Chloromethane is a gas under conditions of normal temperature and pressure. To adequately test such substances in bacterial mutation systems requires special test methods and procedures that were not mentioned in the reported study. The test as described in the Diamond Shamrock submission is a spot test. In a spot test, bacteria are incorporated into top agar and poured over a base plate of minimal medium. The test agent is then placed on the plate (either in crystalline form or on a liquid saturated filter paper disc) and allowed to diffuse into the medium. The formation of a ring or concentrated zone of mutant colonies in the vicinity of the test sample is generally considered to be a positive result. The report submitted by Diamond Shamrock states that 1 to 5 ug of test chemical (chloromethane was one of a series tested) were added to the plate with a spatula. No discussion of the method of testing chloromethane, a gas at normal temperature and pressure, is made. The results of this assay are open to question because the spot test, as described, is inappropriate for testing chloromethane. In addition, the EPA feels that the evidence of a single negative test result conducted under less than optimal conditions is outweighed by the positive results obtained in three independent studies. The EPA, therefore, considers chloromethane to be mutagenic for S. typhimurium strains TA 1535 and TA 100.

Chloromethane was also reported to be inactive in a host-mediated assay in which S. typhimurium strain TA 100 was used as

an indicator organism (Diamond Shamrock 1978a). The host-mediated assay employs an intact mammalian host as the activation system for a microbial mutagen. The test chemical is administered to animals over a period of time which may range from several hours to several days. At the end of the treatment period, the indicator organism is administered to the host animal and allowed to incubate, presumably in the presence of the test agent and/or its metabolites, over a period of several hours. At the end of the incubation period, the indicator organisms are removed and plated on a selective medium to determine mutation. The test chemical may be administered by one of several routes; e.g., intraperitoneally, intramuscularly, intratesticularly, or orally. The indicator organism is generally administered by a different route than the test chemical. The indicator organisms are most often administered by intraperitoneal and intravenous injection. Indicator organisms include bacteria, yeast and some mammalian cells capable of growth in culture. In the study submitted by Diamond Shamrock, the test chemical was administered orally and the indicator organism was administered intraperitoneally. The report states that the test chemical was dissolved or suspended in 10 percent ethanol or peanut oil, without specifying which was used for chloromethane and without specifying how gaseous chloromethane was added to the solvent. Concentrations are given in mg/kg but with no indication of how this was determined. No positive control data were presented; test data which are presented are inadequate and not subject to critical review and evaluation. In addition, the variables

inherent in this system, e.g., concentration of test agent in the animal, animal strain insensitivity, less than optimal amounts of test substance administered, failure of the test chemical or its active metabolites to reach the bacteria in effective amounts, or administration of either test agent or bacteria by the least effective route, may have resulted in false-negative or seemingly incongruous results with chloromethane in this assay. For these reasons, the EPA considers the lost-mediated assay particular test results to be of questionable value in assessing the mutagenic potential of chloromethane.

The EPA believes that in any instance where contradictory data is received on mutagenicity tests, even if all tests are well-conducted, further testing is necessary to determine if a potential human risk exists.

2. Heritable Translocation

Chloromethane has also been reported to cause chromosome breaks in pollen grains of *Tradescantia paludosa* (Smith and Lotfy 1954). At optimal levels for each, chloromethane (9231 ppm) caused a higher percentage of chromatid breaks (240 breaks/5,932 chromosomes, or 4.04 percent) than did ethylene oxide at 7692 ppm (24 breaks/2,150 chromosomes, or 1.12 percent). At equivalent ppm (10,769) chloromethane was also more potent than ethylene oxide in causing chromatid breaks (3.09 percent vs. 0.65 percent). Chloromethane produced only chromatid breaks, however, while ethylene oxide also induced erosions and contractions, leading to a higher level of total chromosomal abnormalities than

chloromethane at optimal levels (5.21 percent vs. 4.04 percent). There were six breaks per 6,590 chromosomes, or 0.09 percent, in untreated control pollen grains. Ethylene oxide is one of the best-studied mutagens known and has demonstrated positive mutagenicity in every system it has been tested in (EPA 1978a).

Diamond Shamrock (1978b) also submitted the results of a rat dominant lethal study in which chloromethane proved to be inactive. A dominant lethal mutation is a change in the germ cell, either egg or sperm, which is lethal to zygotes produced by the mutated germ cell. In mammals, dominant lethal mutations will reduce litter size. This reduction in litter size can be due to the failure of the fertilized egg to implant or to develop after implantation has taken place. Brewen et al. (1975) have shown that dominant lethality results from chromosome breakage, and that the incidence of broken chromosomes at metaphase of the first cleavage of the fertilized egg corresponds to the incidence of dominant lethal eggs. From the tenth day of pregnancy onward in rats and mice, uterine contents can be recognized and classified into living embryos and early and late fetal deaths. Dominant lethal tests can be performed by exposing either male or female animals to the test substance and mating them with untreated members of the opposite sex. The test is most commonly performed by treating male animals and mating them to untreated females. The most common route of administration of the test substance is intraperitoneally; other routes include ingestion, gavage and inhalation. In the test as described by Diamond

Shamrock (1978b), chloromethane was administered by oral intubation as a saturated solution in dichloromethane. Given the gaseous nature of chloromethane, exposure by inhalation is considered to be more appropriate and would have eliminated the need to use a solvent such as dichloromethane which is itself a biologically active material (see Section III.D.3., III.E., and III.F.4.). In any case, a dichloromethane control should have been included in the study and this was not reported. The assay is also difficult to evaluate because of apparent inconsistencies in the data and because of the manner in which the data are presented (Diamond Shamrock 1978b). The test as reported is divided into two parts: an acute and a subacute study. Survival rates of the animals used for the positive control are shown in a table that presents survival data only and are presented later in a table which shows fertility data. The data in the two tables do not agree. The narrative text of the study and the table which presents survival data imply that separate groups of animals were used as negative controls in the acute and subacute parts of the study. The tables which present fertility data imply that the same animals served as negative controls for both parts of the study. Data on such aspects of the study as corpora lutea counts and preimplantation loss, necessary to properly evaluate the results, are not presented and a table listing average implants fails to specify whether it is referring to total implants (live plus dead embryos), or living embryos only. The data as presented are difficult to interpret, and do not lend themselves to statistical evaluation and critical

review. As a result, the validity of the dominant lethal study as presented is open to question.

D. Oncogenicity

Neither epidemiology or other systemic human studies nor any animal assays sufficient to evaluate the oncogenicity of chloromethane have been reported. However, there is substantial information suggesting that this chemical may possess oncogenic potential. This information includes evidence on its mutagenic activity, on its *in vitro* and *in vivo* alkylating capabilities, and on its structural relationship to known or suspected oncogens.

1. Mutagenic Activity

As described earlier, chloromethane has been reported to possess mutagenic activity in bacterial systems that detect gene mutations and to cause chromosomal aberrations in higher plants (see Section III.C. for detailed discussion and evaluation of each of these studies). In assays employing *S. typhimurium* test strains TA 1535 and TA 100, the chemical induced a strong, positive dose-dependent mutagenic response, both with and without metabolic activation (Andrews et al. 1976, DuPont 1978, Simmon 1977). These tester strains detect base pair mutagens. In *Tradescantia paludosa* pollen tubes, chloromethane increased the chromatid breakage rate about forty-fold (Smith and Lotfy 1954). Considering chloromethane's activity in *S. typhimurium* strains TA 100 and TA 1535 and in *Tradescantia paludosa* pollen

tubes, the EPA considers chloromethane to be a direct acting mutagen (i.e., it does not have to be metabolized to be active).

The concept that neoplasms arise from mutations in somatic cells was originally postulated by Boveri in 1914 to account for the unlimited variety of tumor types, and the fact that, on cell division, the daughter cells maintain their neoplastic properties (Boveri 1914, Chu et al. 1977, Trosko and Chang 1978). Oncogens and mutagens have two properties in common: 1) the ability to induce new properties in cells that can be transmitted to their daughter cells; and 2) the ability to convert normal cells into irreversibly changed cells (Suss et al. 1973). Although the mutation theory of oncogenesis is still waiting for unequivocal experimental proof, the theory has recently gained more attention because of three important findings. First, in the 1960's, the Millers at the University of Wisconsin discovered that the majority of oncogens need to be metabolized in order to be active (Miller and Miller 1974, Miller 1978, Miller 1979); second, *in vitro* metabolic activation systems which could be incorporated into mutagenicity assay systems were developed (Malling and Chu 1974); and third, comparison of the ultimate reactive metabolites of structurally diverse oncogens and mutagens revealed that the common denominator of these substances is their electrophilicity, (i.e., they are compounds whose atoms have an electron deficiency that enables them to react with electron-rich sites in cellular nucleic acids and proteins) (Bartsch 1976, Miller 1979). These three findings have now been verified by a host of experimental data which show that oncogens can induce different types of

mutations including gene mutations (both base pair substitution and frameshift alterations), chromosomal aberrations, and non-disjunctions. The oncogenic potential of a chemical has also been correlated with its ability to interact with and modify DNA (Rosenkranz and Poirier 1979).

A wide variety of assay systems have been developed to detect effects on genetic material, including gene mutations and chromosomal aberrations. Gene mutations are alterations in part(s) of the DNA and may be due to substitutions, loss, or acquisition of one or more pairs of nucleotides. Such mutations may be detected as forward (i.e., a change from the wild type) or backward (i.e., reversion to wild type in a mutant strain) mutations.

The Ames Test is an assay that measures gene mutation at histidine loci in Salmonella typhimurium (a bacterium) (Ames et al. 1975). Five mutant strains, which can measure backward base pair substitutions or frameshift mutations, are treated with the test chemical, and histidine revertants are selectively cultured from a population of histidine requiring cells by their ability to grow on media without histidine. Tests are performed both with and without metabolic activation.

The Ames test is a relatively easy test for detecting mutagens. Advantages of the Ames test are that the tester strains are well characterized, thus ensuring that gene mutation is the characteristic being measured and indicating the type of mutational event being caused. The disadvantages, however, are that reverse rather than forward mutation is measured, a

prokaryotic, not a eukaryotic system is measured, and the test is *in vitro*.

The particular value of the Ames test to the EPA's work is that it can be used as a indicator of oncogenic potential. An excellent correlation between mutagenic activity and oncogenic activity has been demonstrated (Bartsch 1976, Brusick 1979). Eighty to ninety percent of the known oncogens tested in this system have been positive. The number of false positives is also low in this system, ranging from 10 to 15 percent.

Chromosomal aberrations are alterations in the structure or number of chromosomes. Structural alterations are mainly the result of breaks in chromosome strand(s) and may lead to unstable nontransmissible changes (achromatic breaks, achromatic gaps, chromatid breaks, chromatid interchanges, acentric fragments, ring chromosomes or dicentric chromosomes) or stable transmissible changes (inversion, translocation, or deletion). Variations in the number of chromosomes, polyploidy or aneuploidy, are mainly the result of anaphase delay, metaphase arrest, endoreduplication, or non-disjunction during mitosis and meiosis. Such chromosomal changes may be detected by cytologic or genetic methods in micro-organisms, plants, insects, mammalian cells *in vitro*, and mammals *in vivo*. While tests on microorganisms largely detect changes in chromosome number, tests on plants, insects, mammalian cells *in vitro* and mammals *in vivo* will detect changes in both chromosome number and structure.

Although chloromethane has been shown to cause chromatid breakage in *T. paludosa* pollen tubes, there are no correlation

studies showing the exact relationship between mutagenic activity in this system and activity as an oncogen.

2. Alkylating Capabilities

Alkylating agents belong to the larger class of reactive compounds called electrophiles (electron seeking). Representative animal tests show that some members of virtually all classes of alkylating agents are oncogenic (Lawley 1976). The chemical basis for their biologic effect is the chemical modification of cellular DNA by these agents (Singer 1975). Lawley (1976) has reviewed oncogenesis by alkylating agents, while Singer (1975) and Pegg (1977) have reviewed the effects of these chemicals on nucleic acids and the relationship of these effects to oncogenesis and mutagenesis.

Alkylation is the most common reaction of chloromethane. In this reaction, the methyl group is transferred to a nucleophilic (electron donating) atom of another molecule with simultaneous elimination of chloride ion, to form a new, stable covalent carbon-heteroatom bond; that is, the nucleophilic reactant is alkylated, or in this case methylated. Chloromethane is a strong enough alkylating agent to be used commercially for this purpose, e.g., in the production of tetramethyllead (von Oettingen 1964). The chemical also has been shown to have alkylating activity in both human (*in vitro*) and rat (*in vivo*) tissues (Redford-Ellis and Gowenlock 1971a, Reynolds and Yee 1967), forming primarily S-methylglutathione and S-methylcysteine. Although some compounds are biologically active only after

metabolic conversion to electrophiles, chloromethane can react directly with genetic material, as illustrated by its ability to induce mutagenesis in S. typhimurium without metabolic activation (Section III.C.). In addition, chloromethane may be metabolized to formaldehyde (see Section III.F.4.), another reactive electrophilic compound.

3. Structure-Activity Relationships

Known chemical oncogens comprise a structurally diverse group of synthetic and naturally occurring organic and inorganic chemicals (Miller and Miller 1974, Miller 1979). Although knowledge of the chemical structures of known oncogens currently provides in itself no way of definitively assessing molecular structures of unknown oncogenicity (Fishbein 1977), certain structural criteria for suspecting chemicals of oncogenic activity have been determined (Arcos 1978). Meeting these criteria are halogenated hydrocarbons and alkylating agents. Chloromethane falls into both categories. Its alkylating properties have been discussed in the preceding subsection.

Chloromethane is structurally very similar to other chlorinated methanes (i.e., dichloromethane, CH_2Cl_2; chloroform, $CHCl_3$; and carbon tetrachloride, CCl_4) and of other monohalomethanes (i.e., fluoromethane, CH_3F; bromomethane, CH_3Br; and iodomethane, CH_3I). Of these structural analogues, chloroform and carbon tetrachloride have been shown to be oncogenic in animals while iodomethane and dichloromethane are suspect oncogens. Another suspect oncogen, formaldehyde (CIIT

1979b), is a known metabolite of chloromethane (see Section III.F.4.). The EPA is not aware of any oncogenicity studies on the other two monohalomethanes, fluoromethane and bromomethane.

Carbon tetrachloride produces liver tumors in the mouse, hamster, and rat following several different routes of administration. The International Agency for Research on Cancer (IARC) determined in 1972 that evidence of the oncogenicity of carbon tetrachloride in experimental animals is sufficient to regard the chemical for practical purposes as if it were oncogenic to humans (IARC 1972, Tomatis 1979).

The oncogenicity potential of chloroform has been reported by NCI (NCI 1976). In this study, the chemical was administered in corn oil via stomach tubing five times per week for 78 weeks to female mice at 7 and 4 mg/day and to male mice at 14 and 8 mg/day. Ninety-five percent of the female mice and 98 percent of the male mice in the high dose groups developed hepatocellular carcinomas while 80 percent and 36 percent, respectively, in the low-dose group developed such tumors (controls: females 1 percent and males 6 percent). Male Osborne-Mendel rats receiving 80 mg/day of chloroform for 78 weeks developed a 24 percent incidence of kidney tumors, 70 percent of which were malignant. The EPA has accepted the study as adequate support for the regulation of chloroform in water on the basis of its oncogenic potential (EPA 1978b).

Iodomethane has been shown to induce lung adenomas in strain A mice following intraperitoneal injection (Poirier et al. 1975)

and to cause local sarcomas with lung metastasis in rats following subcutaneous injections (Druckrey et al. 1970). The development of lung adenomas in strain A mice is considered to be a sensitive indicator of the oncogenic activity of alkylating agents (such as iodomethane) (Poirier et al. 1975, Weisburger 1978). In the case of iodomethane, 0.31 mmole/kg (44 mg/kg) given over a 24-week period (3 times per week) induced a significant increase in the average number of lung adenomas per mouse. In fact, iodomethane on a mmole basis was more active than urethane, which is the usual positive control used in this assay system. In the Druckrey et al. studies (1970), all 6 rats receiving 20 mg/kg of iodomethane once a week for a year developed sarcomas at the site of injection. Of 12 rats receiving 10 mg/kg once a week for a year, 11 developed sarcomas at the site of injection. Another important finding was that in most cases the tumours had metastasized to the lungs. The latter information indicates the malignant nature of the induced tumours. Although neither of these studies provides sufficient information on iodomethane oncogenicity to do an adequate hazard assessment, they do indicate that it has oncogenic potential.

While evidence on the oncogenic potential of dichloromethane is inconclusive because of the inadequacy of the data reported (Theiss et al. 1977, Rampy et al. 1979), it nevertheless has been demonstrated that dichloromethane can cause a statistically significant increase in the numbers of benign mammary tumors in female Sprague-Dawley rats at levels of 500-3500 ppm and in males at 3500 ppm (Rampy et al. 1979).

The EPA has recently received from CIIT a notice of the development of nasal squamous cell carcinomas in rats exposed to formaldehyde (a metabolite of chloromethane), at 6 and 15 ppm (CIIT 1979c). Although the study is not complete and signficance of these findings cannot be fully assessed, the discovery of such rare tumours in this species leads the EPA to consider the preliminary notification of great import. Exposure to formaldehyde was by inhalation and the carcinomas were found in the nose, so that the irritant effect and localized high levels may play an important part in the oncogenicity, whereas the production of formaldehyde as a metabolite of chloromethane might lead to different results, since concentrations would be expected to be diffuse.

E. Teratogenicity

Little direct information is available regarding the teratogenic potential of chloromethane. There has been one report associating the birth of a severely deformed child with maternal exposure to chloromethane and ammonia vapors (Kucera 1968), but no details as to dose, length, or time of exposure are presented. To date no animal studies to evaluate the effects of chloromethane on the fetus have been published. However, Smith and von Oettingen (1947a) reported that "a rabbit conceived and born during exposure to 500 ppm grew at a normal rate during 33 weeks of exposure," but developed slight neuromuscular symptoms like those seen in adults. Because exposures continued after

birth, and only one rabbit was so exposed, no conclusion can be drawn from this study.

Chloroform (trichloromethane) has been found by Schwetz et al. (1974) to be both embryotoxic and fetotoxic. Serious malformations, as well as retarded fetal development, were reported. In addition, Murray et al. (1979) observed a statistically significant increase in cleft palate in mice due to chloroform exposure. Although cleft palate was not observed in a longer dosing regimen, embryotoxicity has now been observed in both the mouse and the rat exposed to chloroform.

Chloromethane, as a gas, would be expected to cross the placenta readily (Villee 1971, Nishimura and Tanimura 1976). Although no direct evidence of chloromethane induced fetal toxicity has been found, Hartman et al. (1955) tell of a seven-month pregnant woman severely poisoned by chloromethane. When found, the fetus had been aborted and was still attached, dead, to the undelivered placenta.

Additional concern for the teratogenic potential of chloromethane is based on its documented neurotoxicity. The central nervous system appears to be especially susceptible to toxic insult during its development (Buelke-Sam and Kimmel 1979). The period during which the CNS develops is an extended one and vulnerability to toxic insult continues into the post-natal period. The possibility of fetal exposure to a neurotoxicant such as chloromethane warrants its evaluation as a teratogen. Evidence has been presented that suggests that both structural and behavioral deficits in adult and developing

systems are associated with exposure to other nonspecific CNS depressant chemicals (van Stee 1976). Few purely behavioral teratogens are known at this time, but psychotropic drugs which have little or no structural teratogenic potential have been identified as behavioral teratogens (Vorhees et al. 1979a). Recent evidence from Bornschein et al. (In Press) indicates behavioral defects in rats exposed in utero to dichloromethane at a dose which caused no morphologic defects (Hardin and Manson In Press).

On the basis of chloromethane's neurotoxicity in adults, accessibility to the fetus, the embryo-fetal effects of its congeners, dichloromethane and chloroform, and in agreement with the concept that anatomical and behavioral evaluations are complementary approaches to CNS toxicity (Barlow and Sullivan 1975, Langman et al. 1975), the EPA concludes that chloromethane has a potential risk for teratogenicity in the human for both behavioral and structural malformations.

F. Metabolism

Although fragmentary research has been accomplished in several areas of chloromethane metabolism, insufficient information exists to give a complete characterization. It is known that chloromethane is absorbed through the lungs, that radioactivity can be detected to varying degrees in all tissues tested following inhalation of ^{14}C-chloromethane, and that excretion of unchanged compound is through the lungs, urine and feces, while possible metabolites also appear in the expired air

and urine. Some percentage of inhaled radioactivity is retained by the organism following administration of $^{14}CH_3Cl$, and is apparently primarily bound to tissue sulfhydryl groups. Other known metabolic products include methanol, formaldehyde, and formate. Although the EPA feels that metabolism studies on chloromethane are not complete, the Agency believes that the data available are sufficient at this time to assist in evaluating the risk of exposure to chloromethane.

1. Absorption

Although it is generally believed that the principal route of human exposure to chloromethane is almost certainly by inhalation, most inhalation experiments in both man and animals are really whole-body exposure experiments and possible skin and GI absorption cannot be wholly ruled out (CIIT 1979d, Smith and von Oettingen 1947a, Stewart et al. 1977, Yevtushenko 1967). It has been demonstrated, moreover, that chloromethane can be absorbed through the skin (NIOSH 1977). However, in one experiment (Morgan et al. 1970) the human volunteers inhaled the radiolabeled chloromethane directly through a tube placed in the mouth (which does not eliminate the possibility of GI or mucous membrane absorption, but does eliminate that through the skin) and showed that absorption through the airways probably does occur.

2. Distribution

Several different experimenters have followed blood and tissue levels of chloromethane over time. The experiments can usually be divided into two types: 1) those in which after a single brief exposure, either by injection or inhalation, the disappearance of chloromethane from the tissue is followed; and 2) those in which the subject has been exposed for a considerable period of time (i.e., the condition is more or less stabilized) and the levels of the chemical are followed after cessation of exposure.

Sperling et al. (1950) injected chloromethane into dogs intravenously (i.v.) and measured blood and tissue chloromethane at various times. At time zero, the percentage of chloromethane present in the blood varied between 4.5 and 13.1 percent (of 1680 mg injected), at 30 minutes, the values were 1.5 to 2.7 percent, and at 60 minutes they ranged from 0.6 to 1.3 percent. However, between eight and thirty minutes were required to inject the total amount of chloromethane (as a gas), thus allowing quite a bit of time for inital redistribution to tissues and biotransformation. This group did another series of experiments where they measured blood and tissue levels in one group of animals at zero time following injection of various amounts of chloromethane (173-206 mg/kg), and another group (154-228 mg/kg) after one hour. The blood chloromethane concentration initially varied between 0.119 to 0.135 mg/cc, while at one hour, the range was 0.035 to 0.041 mg/cc. Neither of the groups showed a dose-blood level relationship, however. Various tissues were measured for

chloromethane content during the same experiments with similar results occurring. Levels at 60 minutes were on the average lower than those at the beginning, although there was considerable variation among dogs.

Soucek (1961) used subcutaneous (s.c.) injection in rats to measure the disappearance of chloromethane from the blood. At two minutes following a single 1200 ug injection of chloromethane in H_2O, 1.4 percent of the dose appeared in the blood, at 10 minutes 0.7 percent, while at 25 minutes, chloromethane concentrations were below the level of detection. However, it is difficult to compare the two experiments, as Soucek could not or did not measure the rate of chloromethane's entrance into the blood, and measurements made after s.c. injection are the result of a two-way flow, both into and out of the blood. Although Soucek was unable to measure chloromethane in the blood beyond 10 minutes, he was still able to detect unaltered chemical in the expired air at 120 minutes, as were Sperling et al. (1950).

CIIT's study (1979d) is of the second type, but with an additional change. Instead of measuring chloromethane, CIIT administered $^{14}CH_3Cl$ and measured radioactivity, which enabled the investigators to pick up metabolites and bound compound as well as free compound dissolved in the plasma. Blood ^{14}C-levels were measured at intervals following a 6-hour exposure to 1500 ppm in rats. At time zero, the ^{14}C-content of the blood was 0.93±0.02 umole of CH_3Cl equivalents/ml, after which the level steadily dropped until at 24 hours the value was 0.17±0.02 umole CH_3Cl equivalents/ml. Levels in all other tissues measured

(liver, fat, kidney, spleen, lung, heart and brain) acted in a similar manner. However, some tissues lost radiolabel much more quickly than others. At 24 hours the amount of ^{14}C in fat was 12.1 percent of the initial value, while the heart still carried 38.9 percent of its initial load. At time zero, liver was highest with 2.63, while brain had the lowest concentration, 0.55 umole CH_3Cl equivalents/ml, but at 24 hours levels in all the tissues were closer to each other, from liver with 0.45 to brain with 0.12 umole CH_3Cl equivalents/g wet weight. There is apparently little or no redistribution to other tissues nor are the organs which appear to be most affected (i.e., brain, liver, kidney) those which retain the greatest amounts of the radioactivity.

3. Excretion

Some portion of the gas is excreted unchanged, not only through the lungs in man (Morgan et al. 1970, Stewart et al. 1977), dogs (Sperling et al. 1950), and rats (Soucek 1961), but in the urine and bile following i.v. injection in the dog (Sperling et al. 1950). CIIT (1979d) also claimed that a portion of the administered chemical was found in the expired air (radiolabel trapped in a charcoal filter but not chemically identified). Under the conditions of the study (6-hour inhalation of different dose levels by rats and mice, 48 hour observation period) the amount excreted unchanged was fairly small and did not appear to be strictly dose-dependent. In rats, at 100 ppm, 2.4 percent of the retained material was found in the

expired air, at 375 ppm, 1.8 percent, and at 1500 ppm, 6.3 percent; in mice, at 1500 ppm, 4.4 percent was exhaled (CIIT 1979d).

Sperling et al. (1950), following injections of various amounts of chloromethane into dogs, observed that about 5 percent of the total injected was found unchanged in the expired air within the first hour. However, Morgan et al. (1970) found that one hour after inhalation of $CH_3{}^{38}Cl$ in man, 29 percent of the administered radioactivity was excreted through the lungs. The EPA believes that all the radioactivity measured in the expired air was chloromethane, rather than a metabolite, even though this was not verified chemically, as all the known or postulated biotransformation mechanisms produce chloride ion, a non-volatile product. The rather large difference between these two results may be due to the mode of administration, to the species, or to what was measured in the expired air: chloromethane in the first case (Sperling et al. 1950) and ^{38}Cl in the second (Morgan et al. 1970).

Morgan et al. (1970) compared the pulmonary excretion of $CH_3{}^{38}Cl$ with that of the higher chlorinated methanes, and concluded that chloromethane acted in a different manner. When excretion rate versus time was plotted (retention curve), the di-, tri- and tetrachlorinated methanes had parallel slopes, while monochloromethane's slope was steeper, cutting across the others. This may be due to a number of reasons: 1) the compound is more reactive; 2) a greater percentage is excreted by alternate routes; 3) it is more fat-soluble. The authors felt

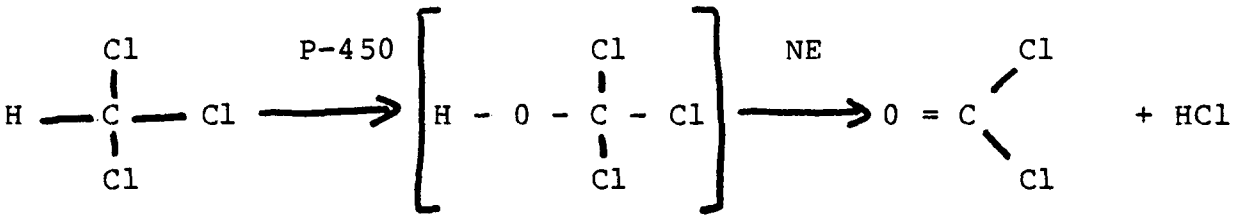
that chloromethane behaved like iodomethane, which reacts rapidly with sulfhydryl groups in the erythrocyte in an enzyme-catalyzed methylation process.

Stewart et al. (1977) discovered two populations among their human subjects. Four of their subjects, as well as two from a previous study, had considerably elevated post-exposure breath and blood chloromethane levels. The rest of the volunteers carried a two to six times lower body burden than these. Stewart and his coworkers postulated that the worker who carries a lower body burden than the majority may be at greater risk from chloromethane exposure. This would appear to indicate that a larger portion of the exposed population would be at greater risk. The data can be interpreted to mean that those people with greater amounts of unaltered compound in their bloodstream and airspace might be metabolizing less of the material rather than absorbing or carrying more. And as the metabolized material is probably responsible for toxicity, the subjects excreting more compound unchanged would be at a lower risk rather than a higher one. If, on the other hand, chloromethane *per se* is the toxic compound, those persons with higher blood and breath levels may be more susceptible to overexposure. Of course, the possibility exists that neither of these theories is important, and the different populations are at equal risk.

4. Biotransformations

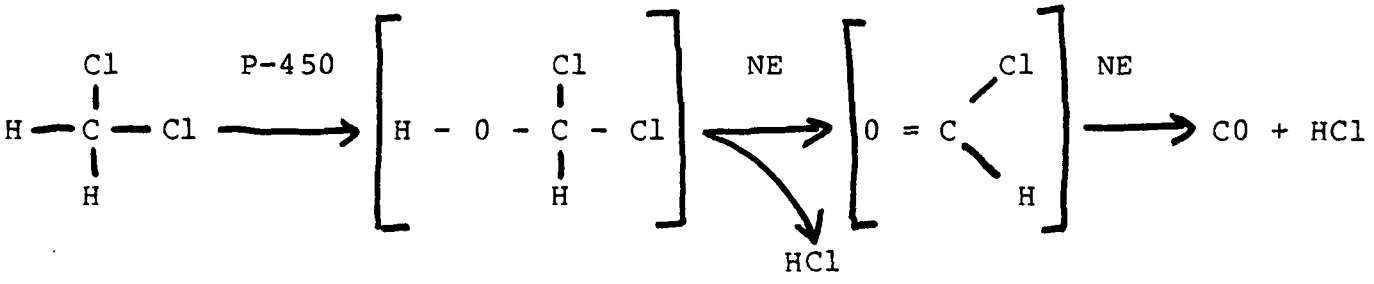
The earliest theories about chloromethane's biotransformation (Flury 1928) dealt with its probable conversion to formaldehyde through methanol. Formaldehyde has been found in the blood of rats (Yevtushenko 1967) and mice (Sujbert 1967) following inhalation of chloromethane, and in mice (Sujbert 1967) following intraperitoneal (i.p.) injection. Sujbert (1967) also was able to detect methanol in the bloodstream of mice following inhalation or i.p. injection as did Hayhurst and Greenburg (1929) who detected methanol, formaldehyde and formates in the organs of victims of chloromethane poisoning. Smith (1947), on the contrary, was unable to find any methanol in the blood of dogs that had been exposed to chloromethane by inhalation for 23 or 25 days. Other researchers have tested for formate in the urine or tissues of subjects exposed to chloromethane, with variable results. Baker (1927), Kegel et al. (1929) and Hayhurst and Greenburg (1929) found formates in human tissues and urine following accidental exposure to the compound, whereas neither Lundgren (1947) nor Hansen et al. (1953) were able to demonstrate increased formate in their human subjects correlating with levels of chloromethane in the ambient air. Lundgren could not establish a correlation between excretion of formate in rabbits, rats, or guinea pigs, and extent of exposure to chloromethane, but guinea pigs in the apartment of a person who died of chloromethane toxicity when her refrigerator sprang a leak, showed formate in their tissues (Hayhurst and Greenburg 1929).

The formation of formaldehyde from chloromethane is probably analogous to that proposed for the biotransformation of chloroform ($CHCl_3$) to phosgene, a reaction that has recently been confirmed experimentally (Pohl et al. 1977, 1979, Pohl and Krishna 1978).

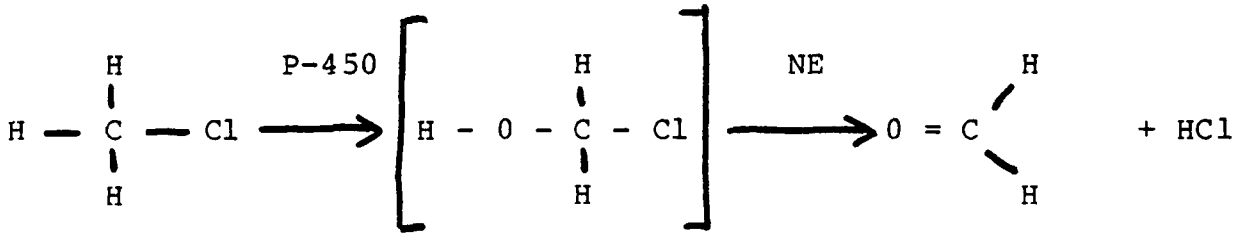


NE = nonenzymatic

It has been suggested that a cytochrome P-450 monooxygenase oxidizes $CHCl_3$ to unstable trichloromethanol, which spontaneously dehydrochlorinates to yield the reactive phosgene. Dichloromethane seems to follow a similar initial pathway (Kubic and Anders 1978) to eventually yield CO through a formyl halide intermediate:

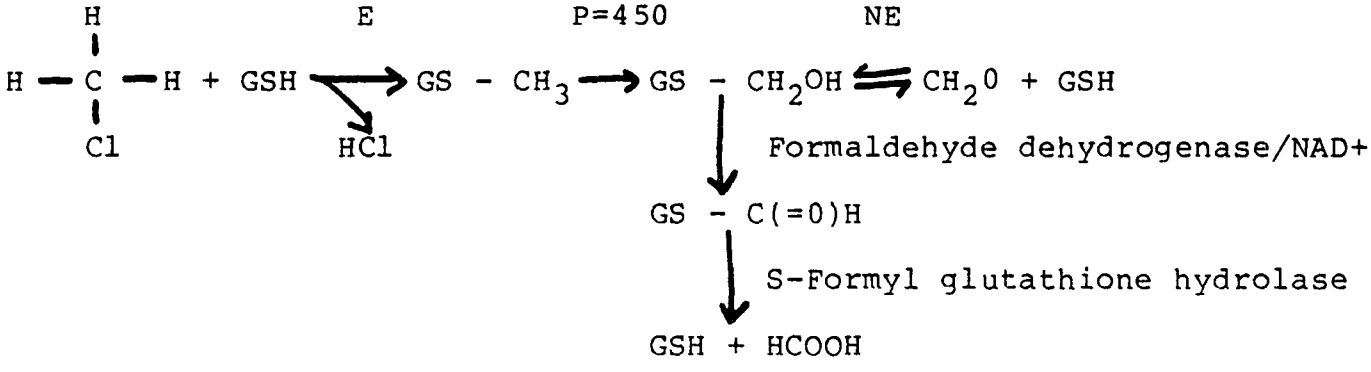


The formation of formaldehyde from chloromethane probably occurs as follows:



Additional reactions can occur after formaldehyde production: (1) aldehyde reduction, with formaldehyde going to methanol; (2) aldehyde dehydrogenation, with formic acid and/or formates as the ultimate product.

Ahmed and Anders (1978) have proposed an additional route for metabolism of dihalomethanes, which involves alkylation and dealkylation of glutathione (GSH). This pathway yields formaldehyde, formic acid and inorganic halide. As it is known that chloromethane binds very specifically to GSH in erythrocytes (Redford-Ellis and Gowenlock 1971a), this alternative route may also be important for chloromethane.



= enzymatic

CIIT (1979d) measured radioactivity in the expired air for 48 hours following a 6-hour exposure to chloromethane in rats and mice. That percentage of radioactivity trapped by a charcoal filter they designated as chloromethane, while that trapped by ethanolamine in methoxyethanol was considered to be CO_2. No mention was made as to whether such possible alternative volatile metabolites as carbon monoxide or formaldehyde would be trapped and measured by their methods.

CIIT (1979d) found that in rats in the 48 hours following a 6-hour exposure to 1500 ppm $^{14}CH_3Cl$, more than 41 percent of the total recovered radioactivity was $^{14}CO_2$ from the expired air, while in mice given the same dose, less than 18 percent was excreted as $^{14}CO_2$. In mice, the largest percentage of recovered radioactivity (60 percent) was excreted in the urine, while the rat excreted only 40 percent of the retained radioactivity as urinary components. It is possible that a portion of the urinary radioactivity occurs as bicarbonate, for following acid hydrolysis of the mouse urine, a small portion (9 percent) of the urinary radioactivity was found in the headspace of the vial, presumably as $^{14}CO_2$.

5. Tissue Retention

In addition to measuring radioactivity in the various excreta, CIIT (1979d) measured radioactivity retained in some organs and the carcass following exposure to $^{14}CH_3Cl$. In rats at the end of a 48 hour period following 6 hours of exposure to 100,

375 or 1500 ppm of the gas, there was no increase in the amount of associated radioactivity between the 375 and 1500 ppm groups (e.g., for liver at 100 ppm, tissue radioactivity equalled 52.3 umole of ^{14}C-chloromethane equivalents/g wet weight, while at 375 ppm, the radioactivity was 325.2, and at 1500 ppm, it was 265.2), which may indicate a saturation of available binding sites. Of the amount retained following a 6 hour exposure, 22.5 percent was associated with the tissues at 100 ppm, 21.4 percent at 375 ppm and 17.3 percent at 1500 ppm. Although neither the form nor the type of binding in the tissues was specified by CIIT, the retention of such a high proportion of radioactivity after two days indicates a fairly strong binding capacity. At all dosages, retention was lowest, by a factor of three, in the brain, highest in the liver at 375 and 1500 ppm and highest in fat at 100 ppm. In mice following a similar exposure regimen at 1500 ppm, only 8.3 percent of the total recovered was associated with the tissues, and while the brain level had again the lowest value, liver and kidney had the highest.

6. Binding

Morgan et al. (1970) postulated that chloromethane acts like iodomethane, reacting rapidly with sulfhydryl groups in an enzyme-catalyzed methylation process. Redford-Ellis and Gowenlock (1971a,b) studied the reaction of ^{14}C-chloromethane with human blood *in vitro*. In serum or plasma, about 65 percent of the radioactive uptake was associated with plasma protein but only about 2-3 percent covalently bound to the plasma protein

(specifically albumin), producing primarily S-methylcysteine, although minor radioactive components of 1-methyl and 3-methylhistidine were also found. In erythrocytes, uptake was independent of dose over the range used (600-1000 mg/ml erythrocyte), being 357 mg/ml erythrocyte after 80 minutes, of which 58-130 mg was bound covalently to glutathione (GSH). However, in studies on red cells, after uptake was complete, no radioactivity was lost by hemolysis or by washing and no radioactivity could be detected bound to any other components of the erythrocyte, so there appears to be some discrepancy between uptake and binding. Heating the blood before adding the chloromethane reduced binding by over 90 percent, indicating a probable enzyme-catalyzed reaction. Redford-Ellis and Gowenlock (1971b) continued their researches by studying chloromethane's binding to rat brain, liver and kidney homogenates _in vitro_, as these are the organs primarily associated with chloromethane toxicity. In all these tissues, the primary products are ^{14}C-S-MeCys and ^{14}C-S-MeGSH, while in the kidney additional traces of radioactivity were found in methionine. The formation of these compounds in tissue homogenates also appears to be partially enzyme-dependent, as heating the tissues reduced the level of binding.

As part of the CIIT study, Dodd et al. (1979) looked at alterations in tissue sulfhydryl concentrations in rats after acute inhalation exposure to 1500 ppm chloromethane for 6 hours. They found that although changes in total tissue sulfhydryl groups were minimal at all times (0,1,2,4,8,18 hours) after

exposure, non-protein sulfhydryl content was reduced in liver, kidney, lung and blood (most to least) indicating a decrease in free reactive SH groups. At eighteen hours after exposure non-protein sulfhydryl content had returned to control values. However, earlier work by the same group (CIIT 1979d) reported that radioactivity was still present in these tissues 48 hours after exposure. It appears to the EPA that either: 1) significant amounts of CH_3Cl or a metabolite are reacting with non-sulfhydryl groups or 2) rearrangement is occurring.

IV. Current and Planned Testing

The Chemical Industry Institute of Toxicology (CIIT) has in progress a toxicologic evaluation of chloromethane in laboratory animals. The major components of the CIIT program are a pharmacokinetics study, a 90-day preliminary study, teratogenesis-reproduction studies, and a 24-month chronic inhalation toxicity study. Thus far, the pharmacokinetics and 90-day probe study have been completed, and the 24-month chronic toxicity study was initiated in June 1978. The teratogenesis-reproduction studies have not yet begun.

The pharmacokinetics study involved the dosing of ^{14}C-labeled chloromethane to rats (F-344 albino) and mice ($B_6C_3F_1$, hybrid) by inhalation (see Section III.F.) (CIIT 1979d).

The 90-day probe study (CIIT 1979a) involved the inhalation exposure of F-344 albino rats and $B_6C_3F_1$ hybrid mice to various levels (300, 750, 1500 ppm) of chloromethane, 6 hours per day, 5

days per week for 13 weeks. There are certain deficiencies associated with this study including the following:

(1) The rat is not the most appropriate test species for systemic chronic effects at the dose levels used. This species was previously shown to be unaffected by exposure to 1000 ppm chloromethane, 6/hours/day, 6 days/week for 64 weeks (Smith and von Oettingen 1947 a,b). Also, since the toxicity of chloromethane decreases as the exposure-free period is increased, one would anticipate that decreasing the exposure frequency to 5 days/week over 90 days would lead to little, if any, toxicity even at 1500 ppm. Dogs or monkeys may be more appropriate since they showed signs of toxicity even at 500 ppm, 6 hours/day, 6 days/week and exhibited neurologic and behavioral effects seen in humans exposed to chloromethane (Smith and von Oettingen 1947a, Smith 1947).

(2) Although the protocol required twice daily observations of the animals to detect signs of toxicity and behavioral changes, no information on these observations, if actually done, was reported.

(3) Of the 80 mice used in the study, 19 died during the 13-week study; 14 of which died due to stated problems with new cages. Seven of those dying of

trauma were in the high dose group of male mice. Although there was no statement of time of death, three of the male mice in the high dose group dying of trauma were necropsied and the results included in the overall histopathologic findings.

(4) Although there were eye lesions in 13 of the treated mice and although chloromethane is known to induce eye lesions (see Section III. B.), there was no clear explanation of the nature of the lesions or why the results were concluded not to be compound related.

(5) There was a wide variability of response in the control groups. A large standard deviation in a control group means that the difference between a treated group and the control group needs to be larger in order to detect a significant difference. Therefore, in a better controlled study, perhaps more significant differences would have been detected.

CIIT (1979b) also has in progress a 24-month chronic inhalation study in mice and rats. This study was initiated in June 1978; to date, the EPA has received a 6 month interim report (CIIT 1979e). The exposure levels being administered are 50, 225, and 1000 ppm. The frequency of administration is 6 hours/day, 5 days/week for 18 months. As planned, during the last 6 months of the study, the animals will not be exposed to

chloromethane but will be held for observation. (CIIT may choose to extend the dosing period to the full 24 months.) There are 120 animals of each sex in each of the three exposure groups and in each of two control groups. Interim sacrifices are scheduled at 6, 12, and 18 months. In the interim report submitted to EPA (October 1979), it was revealed that female mice in all treated groups and male mice treated with 1000 ppm showed significant body weight decrements compared to controls. This is in contrast to the results reported in the 90-day probe study. Chronic inhalation of chloromethane in mice (1000 ppm) was reported to be associated with focal acute scleritis (3/10 males, 1/10 females), hepatocellular degeneration (7/10 males, 7/10 females), splenic lymphoid depletion (8/10 males, 4/10 females) and thymic lymphoid necrosis (4/10 males, 1/10 females). In rats chronic administration of the chemical was reported to be associated with sperm granuloma (2/10), interstitial pneumonia (1/10 males, 4/10 females) and subacute tracheitis in females (5/10). No significant histopathologic findings were discovered in the liver of rats or in the kidneys of rats or mice.

There are several difficulties with both the design and the execution of the 24 month study that CIIT has started:

1. In the six-month interim report, CIIT reported a significant death rate due to trauma, especially within the first 6 months in male mice. There was an overall death rate due to trauma of 4.5 percent. For male mice this rate was almost 9 percent. If this

rate continues over the next 18 months of the study, there will not be sufficient animals surviving to evaluate.

2. In the 90-day study (CIIT 1979a), there was no significant weight decrement in treated male mice even at 1500 ppm, while female mice showed such a decrement only at 1500 ppm. Under these circumstances, the reason for the choice of 1000 ppm as the high dose in this species for determining either oncogenicity potential or chronic toxicity is unclear. However, the 6-month interim report weight loss has been recorded in all groups of treated female mice. The EPA believes that in an oncogenicity study, no toxic effects should be seen in the two lower dose groups other than tumors, in order to ensure the survival of enough of the animals to demonstrate the production of tumors as a late effect. On the other hand, if considered as solely a straightforward chronic toxicity test, the EPA prefers that the lowest dose represent a no-effect level, i.e., demonstrate no toxicity at such an early stage, even weight loss.

Regarding chronic toxicity specifically, various differences from the EPA's proposed protocol (EPA 1979c) raised questions concerning the usefulness of the results:

1. As discussed previously in Section III.A.2., use of rats have been exposed at levels for which rats had previously been shown to exhibit no effects on the liver, kidney, cardiovascular or hematopoietic systems. CIIT's original protocol (CIIT 1979b) indicated that it was originally planning on using dogs as well as rats and mice for the 24-month inhalation study. CIIT has not given the EPA any reasons for this change in protocol. In this particular case the lack of a non-rodent species as required by the EPA for chronic toxicity testing is more than usually important. Because non-rodents such as dogs and monkeys appear to be more sensitive to chloromethane, the use of rats and mice may give spurious no-effect levels when used to evaluate the risk to humans.

2. There is no indication that the animals were adequately observed during the first 6 months for general toxicity or for neurologic or behavioral toxicity.

3. No tests will be conducted to determine the more discriminating aspects of behavior and performance.

The Agency believes that because the information gathered as a result of this study, along with the previous data available on

chronic systemic effects other than neurologic, will be adequate for hazard assessment purposes for systemic effects other than neurotoxicity, it is not deemed necessary to require further general chronic toxicity testing. The EPA will be requiring neurobehavioral studies to evaluate risk from the apparently most sensitive system.

Regarding oncogenicity, the EPA has concluded that the eighteen-month exposure period may not be sufficient to indicate a lack of oncogenicity. If no tumours are detected in the test animals, the Agency has decided that a minimum of 24 months in a rodent is necessary to demonstrate a lack of oncogenicity for the Agency's purpose (EPA 1979e).

This deficiency, in addition to those discussed earlier under general problems, is severe enough to warrant additional testing, as there is at present no way to determine the oncogenic potential of a chemical other than by adequate long-term testing.

Although CIIT has not yet initiated its teratology testing, they have proposed a protocol for teratologic evaluation of chloromethane (CIIT 1978f). Using this protocol, CIIT intends to collect data on anatomical abnormalities, neurofunctional deficits, and acquisition of developmental landmarks in rats exposed to chloromethane *in utero*.

The CIIT protocol differs significantly from that proposed by the EPA (EPA 1979b) in several ways:

(1) It specifies the use of a single species, the rat, to evaluate teratogenic effects. The EPA has proposed teratology testing in a minimum of two mammalian species. A study in one with negative results would be considered inadequate, although findings of malformations in a single species would be highly suggestive of teratogenesis.

(2) The dosages proposed are arbitrary and do not conform to suggested criteria for dosage levels as published in the proposed Test Standards (EPA 1979b). The intermediate dose should be related to the high dose (which should cause toxicity) not with the lower dosage, as indicated in the protocol. This is particularly the case where extensive embryonic or fetal death occurs at the high dose. If maternal toxicity occurs at the high dose but no fetal toxicity is demonstrated, then no fetal effects need to be demonstrated in the intermediate dosage range. The EPA believes that the low dose of 100 ppm or some multiple thereof is satisfactory.

(3) Although the battery of tests for the evaluation of neurofunctional deficits and the acquisition of developmental landmarks proposed by CIIT (1979b) may not be completely appropriate, standards for the testing of behavioral alterations have not yet been

proposed by the EPA and the Agency will consider CIIT's proposed battery as the basin for such testing. Guidance will be proposed for public comment in an Advanced Notice of Proposed Rule Making (ANPRM).

(4) Because the rat has demonstrated less sensitivity to chloromethane intoxication than other species (Smith and von Oettingen 1947a), such other species should be considered.

REFERENCES

Anonymous. 1945. Late effects of methyl chloride poisoning. J. Med. Assoc. 127:882.

Aaron CS, Lee WR. 1978. Molecular dosimetry of the mutagen ethyl methanesulfonate in Drosophila melanogaster spermatozoa: Linear relation of DNA alkylation per sperm cell (dose) to sex-linked recessive lethals. Mutat. Res. 49:27-44.

Aaron CS, von Zeeland AA, Mohn GR, et al. In press. Molecular dosimetry of the chemical mutagen ethyl methanesulfonate:Quantitative comparison of mutatin induction in Escherichia coli, V-79 Chinese hamster cells, 5178Y mouse lymphoma cells and some in vitro and in vivo cytological results. Mutat. Res. (in press).

Ahlstrom RC Jr, Steele JM. 1979. In: Standen A, ed. Kirk-Othmer encyclopedia of chemical technology, New York: Interscience Publishers, 3rd ed., vol. 5, pp. 677-685.

Ahmed AE, Anders MW. 1978. Metabolism of dihalomethanes to formaldehyde and inorganic halide. II. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2021-2025.

American Conference of Governmental Industrial Hygienists (ACGIH) 1971. Documentation of the threshold limit values for substances in workroom air. Cincinnati, OH: ACGIH, pp. 160-161.

American Conference of Governmental Industrial Hygienists (ACGIH). 1979. Threshold limit values for chemical substances and physical agents in the workroom environment with intended changes for 1979. Cincinnati, OH:ACGIH.

Ames BN, McCann J, Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31: 347-364.

Andrews AW, Zawistowski ES, Valentine CR. 1976. A comparison of the mutagenic properties of vinyl chloride and methyl chloride. Mutat. Res. 40: 273-276.

Arcos JC. 1978. Criteria for selecting chemical compounds for carcinogenicity testing: An essay. J. Environ. Pathol. Toxicol. 1: 433-458.

Baker HM. 1927. Intoxication with commercial methyl chloride. J. Am. Med. Assoc. 88: 1137-1138.

Barlow SM, Sullivan FM. 1975. Behavioral teratology. In: Berry CL, Poswillo DF, eds. Teratology: trends and applications. New York: Springer-Verlag, pp. 103-120.

Bartsch H. 1976. Predictive value of mutagenicity tests in chemical carcinogenesis. Mutat. Res. 38: 177-190.

Belova SF, Yevtushenko GY. 1967. Toxic effect of methyl chloride on the visual organ. Toksikol. Novykh Prom. Khim. Veshchestv 9:182-187. (Russian)

Birch CA. 1935. Methyl chloride poisoning. Lancet I: 259-260.

Borghetti A, Gobbato F. 1969. Acute renal insufficiency from chlorinated derivatives of aliphatic hydrocarbons. Giornale Clin. Med. 50: 463-487. (Italian)

Bornschein RL, Hastings L, Manson JM. In press. Behavioral toxicity in the offspring of rats exposed to dichloromethane (DCM) prior to and/or during gestation. Toxicol. Appl. Pharmacol. (in press).

Boveri T. 1914. Zur frage der entstehung maligner tumoren. Jena: Gusta Fischer. (German)

Brewen JG, Payne HS, Jones KP, Preston RJ. 1975. Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic germ cells. Mutat. Res. 33: 239-250.

Brusick, D. 1979. Bacterial mutagenesis and its role in the identification of potential animal carcinogens. In: Griffin AC, Shaw CR, eds. Carcinogens: identification and mechanisms of Action. New York: Raven Press, pp. 3-105.

Buelke-Sam J, Kimmel CA. 1979. Development and screening methods for behavioral teratology. Teratology 20: 17-30.

Chemical Industry Institute of Toxicology (CIIT). 1979a. A ninety-day inhalation toxicology study in F-344 albino rats and $B_6C_3F_1$mice exposed to atmospheric methyl chloride gas. Battelle Laboratories. 1979. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Industry Institute of Toxicology (CIIT). 1979b. A proposed toxicological evaluation of methyl chloride in laboratory animals. CIIT. 1977. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Industry Institute of Toxicology (CIIT). 1979c. Formalydehyde research findings. TSCA Sec. 8(e) submission 8EHQ-1079-0314. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Industry Institute of Toxicology (CIIT). 1979d. Disposition of 14C-methyl chloride in rats and mice. CIIT. 1979. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Industry Institute of Toxicology (CIIT). 1979e. Interim report on a chronic inhalation toxicology study in rats and mice exposed to methyl chloride (6-month status). CIIT 1979. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Industry Institute of Toxicology (CIIT). 1979f. Tentative protocol for teratologic evaluation of methyl chloride. CIIT. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Chemical Marketing Reporter. (CMR) 1976. Chemical profile-Methyl chloride. p.9. March 29, 1976.

Chemical Marketing Reporter (CMR). 1979. Chemical profile - Methyl chloride. February 12, 1979.

Cremieux L, Herman JA. 1974. Photolysis of gaseous ethyl chloride below and above the ionization potential. Can. J. Chem. 52:3098-3105.

Cronn DR, Rasmussen RA, Robinson E, Harsch DE. 1977. Halogenated compound identification and measurement in the troposphere and lower stratosphere. J. Geophys. Res. 82: 5935-5944.

Chu EHY, Trosko JE, Chang CC. 1977. Mutational approaches to the study of carcinogenesis. J. Toxicol. Environ. Health 2: 1317-1324.

DeForest EM. 1979. Chloromethanes. Encycl. Chem. Process. Des. vol. 8. New York: Marcel Dekker, Inc. pp. 214-270.

Del Zotti G, Gillardi V. 1954. Hepatic function in workers of the refrigerating industry exposed to the inhalation of gases. Med. Lavoro 55: 350-356. (Italian)

Diamond Shamrock. 1978a. Chloromethane. In vitro and subacute in vivo host-mediated assay for mutagenesis. Bio/Tox. TSCA Sec. 8(d) submission 8DHQ-1078-0198. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Diamond Shamrock. 1978b. Chloromethane. Dominant lethal study in rats. Bio/Tox. TSCA Sec. 8(d) submission 8DHQ-1078-0196. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Dodd DE, Bus JS, Barrow CS. 1979. Alterations in tissue sulfhydryl concentrations in rats after acute inhalation exposure to methyl chloride. Pharmacologist 21:215.

Drake JW. 1975. Environmental mutagenic hazards. Science 187: 503-514.

Druckrey H, Kruse H, Preussmann R, Ivankovic S, Landschutz C. 1970. Cancerogenic alkylating substances. III. Alkylhalide, -sulfate, -sulfonate and ring strained heterocyclic compounds. Z. Krebsforschung 74: 241-273. (German)

Dunn RC, Smith WW. 1947. Acute and chronic toxicity of methyl chloride IV. Histopathologic observations. Arch. Pathol. 43: 296-300.

DuPont. 1978. Chloromethane mutagenicity test. Haskell Laboratory. 1977. TSCA Sec. 8(d) submission 8DHQ-1078-0204. Washington DC: Office of Toxic Substances, U.S. Environmental Protection Agency.

Fishbein L. 1978. Structural parameters associated with carcinogenesis (halogenated olefins, vinyl and allyl analogs and epoxides). In: Asher IM, Zervos C, eds. Structural correlates of carcinogenesis and mutagenesis. A guide to testing priorities. Washington DC: Department of Health, Education and Welfare (DHEW Pub FDA 78-1046) pp. 8-21.

Flamm WG. 1977a. Mutagenicity tests. In: Principles and Procedures for Evaluating the Toxicity of Household Substances. Prepared by the Committee for the Revision of NAS Publication 1138. National Academy of Sciences: 86-98.

Flamm WG. 1977b. Approaches to determining the mutagenic properties of chemicals: risk to future generations. J. Environ. Pathol. and Toxicol. 1:301-352.

Flamm WG. 1979. Mutagenicity guideline. (Draft report).

Flury F. 1928. Modern industrial intoxications. Arch. Exp. Pathol. Pharmakol. 138:65-82. (German)

Gaultier M, Frejaville JP, LeBreton R, Garat J, Gervais P, Fournier E. 1965. On the difficulty of diagnosing certain mass poisonings. A case of familial poisoning from methyl chloride. Ann. Med. Leg. 45:273-275. (French)

Gerbis H. 1914. Peculiar conditions of narcosis following industrial use of methyl chloride. Muenchener Med. Wochensch. 61:879. (German)

Gudmundsson G. 1977. Methyl chloride poisoning 13 years later. Arch. Environ. Health 32: 236-237.

Gummert M. 1961. The Wilson block after methyl chloride intoxication. Zeitschr. Ges. Inn. Med. Ihregrenzegebiete 16:677-680. (German)

Hansen H, Weaver NK, Venable FS. 1953. Methyl chloride intoxication. Arch. Ind. Hyg. Occup. Med. 8: 328-334.

Hardin BD, Manson JM. In press. Absence of dichloromethane teratogenicty with inhalation exposure in rats. Toxicol. Appl. Pharmacol. (in press).

Hartman TL, Wacker W, Roll RM. 1955. Methyl chloride intoxication. Report of two cases, one complicating pregnancy. N. Eng. J. Med. 253:552-554.

Harsch D. 1977. Study of halocarbon concentrations in indoor environments. Final report for EPA Contract No. WA 6-99-2922-J. Washington DC: U.S. Environmental Protection Agency.

Hayhurst ER, Greenburg L. 1929. Poisoning from commercial methyl chloride. Am. J. Pub. Health 19:1048-1051.

Interagency Testing Committee (ITC). 1978. Initial report of the TSCA Interagency Testing Committee to the Administrator, Environmental Protection Agency. Washington, DC: U.S. Environmental Protection Agency (EPA 560-10/78-001).

International Agency for Research on Cancer (IARC). 1972. IARC monographs on the evaluation of chemicals to man. 1. Some inorganic substances, chlorinated hydrocarbons, aromatic amines, n-nitroso compounds and natural products. Lyon, France: IARC.

Kegel AH, McNally WD, Pope AS. 1929. Methyl chloride poisoning from domestic refrigerators. J. Am. Med. Assoc. 93: 353-358.

Klimkova-Deutschova E. 1957. Research of the neurological picture in methyl chloride poisoning. Rev. Czech. Med. 3:1-11.

Kubic VL, Anders MW. 1978. Metabolism of dihalomethanes to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2349-2355.

Kucera J. 1968. Exposure to fat solvents: A possible cause of sacral agenesis in man. J. Pediat. 72: 857-859.

Langauer-Lewowicka H, Manowska T, Dobrogowska-Kunicka J. 1974. Assessment of nervous system in persons exposed to the action of organic solvents. Neurol. Neurochir. Pol. 24: 559-564. (Polish)

Langman J, Webster W, Rodier P. 1975. Morphological and behavioral abnormalities caused by insults to the CNS in the perinatal period. In: Berry CL, Poswillo DE, eds. Teratology: trends and applications. New York: Springer-Verlag. pp.182-200.

Laskowski S, Tomczewska-Wilk A, Ratajski W, Pohorski A. 1976. Methylchloride poisoning. Wiad. Lek. 29:59-62. (Polish)

Lawley PD. 1976. Carcinogenesis by alkylating agents. In: Searle CE, ed. Chemical carcinogens. ACS monograph 173. pp.83-244.

Lee WR. 1978. Dosimetry of chemical mutagens in eukaryote germ cells. In: Hollaender A, ed. Chemical mutagens. vol. 5, New York: Plenum Press. pp. 177-202.

Leo A, Hansch C, Elkins D. 1971. Partition coefficients and their uses. Chem. Rev. 71: 525-616.

Lowenheim FA, Moran MK. 1975. Faith, Keys, and Clark's industrial chemicals, 4th ed. New York: Wiley Interscience. pp. 530-538.

Lovelock JE. 1975. Natural halocarbons in the air and in the sea. Nature 256:193-194.

Lundgren KD. 1947. Methyl chloride poisoning in workers in the refrigerator industry. Nordisk Hyg. Tidskr. 28:180-190. (Norwegian)

MacDonald JDC. 1964. Methyl chloride intoxication. J. Occup. Med. 6: 81-84.

Mackie IJ. 1961. Methyl chloride intoxication. Med. J. Australia I: 203-205.

Malling HV, Chu EHY. 1974. Development of mutational model systems for study of carcinogenesis. In: Ts'o, PO, DiPaolo JA, eds. Chemical carcinogenesis. Part B. New York: Marcel Dekker, Inc. pp. 545-563.

McElheny VK, Abrahamson S, eds. 1979. Assessing chemical mutagens: the risk to humans. Banbury Report 1. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

McNally WD. 1946. Eight cases of methyl chloride poisoning with three deaths. J. Ind. Hyg. Toxicol. 28:94-97.

Milkov LE, Popova TB, Soloveva EA, Yevtushenko GY, Lvovskaya EN. 1965. Early functional changes in the body of workers in contact with methyl chloride. In: Occupational diseases in the chemical industry. Moscow: Meditsina. pp. 224-226. (Russian)

Miller EC. 1978. Some current perspective on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Research 38:1479-1496.

Miller EC, Miller JA. 1974. Biochemical mechanisms of chemical carcinogenesis. In: Busch H. ed. The molecular biology of cancer. New York: Academic Press, Inc. pp.377-402.

Miller JA. 1979. Concluding remarks on chemicals and chemical carcinogenesis. In: Griffin AC, Shaw CR, eds. Carcinogens: identification and mechanisms of action. New York: Raven Press. pp.455-469.

Ministry of Health and Welfare (MHW). 1975. The testing of chemicals for carcinogenicity, mutagenicity and teratogenicity. Ohawa, Canada: Ministry of Health and Welfare.

Morgan A, Black A, Belcher DR. 1970. The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann. Occup. Hyg. 13:219-233.

Murray FJ, Schwetz BA, McBride JG, Staples RE. 1979. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol. Appl. Pharmacol. 50:515-522.

National Academy of Sciences (NAS). 1976. Halocarbons: effects on stratospheric ozone. Washington DC: National Academy of Sciences.

National Academy of Sciences (NAS). 1978. Nonfluorinated halomethanes in the environment. Washington DC: National Academy of Sciences.

National Cancer Institute (NCI). 1976. Report on carcinogenesis bioassay of chloroform. Washington DC (NIH 76-1279).

National Institute for Occupational Safety and Health (NIOSH). 1972-1974. National Occupational Hazard Survey Data Base.

National Institute for Occupational Safety and Health (NIOSH). 1977. Occupational diseases. A guide to their recognition. Washington DC: Department of Health, Education and Welfare. DHEW (NIOSH) Publication No. 77-181.

National Institute for Occupational Safety and Health (NIOSH). 1978. Walk-through industrial hygiene survey of Dow Chemical Company, Midland, Michigan.

National Science Foundation (NSF). 1975. Research program on hazard priority ranking of manufactured chemicals. Phase II - final report. Washington DC: National Science Foundation. (PB 263164).

Nishimura H, Tanimura T. 1976. Clinical aspects of the teratogenicity of drugs. New York: American Elsevier, pp. 56-57.

Noro L, Pettersson T. 1960. Methyl chloride intoxication. Nordisk. Med. 14: 381-384. (Norwegian)

Office of Pesticide Programs (OPP), U.S. Environmental Protection Agency. 1978. Proposed guidelines for registering pesticides in the U.S.; hazard evaluation: humans and domestic animals. Federal Register, Aug. 22, 1978, 43 FR 37336-37403.

Office of Toxic Substances (OTS), U.S. Environmental Protection Agency. 1979. Toxic substances control act chemical substance inventory. Washington, DC: U.S. Environmental Protection Agency. 5 vols.

Office of Water Planning and Standards (OWPS), U.S. Environmental Protection Agency. 1979. Halomethanes. Ambient water quality criteria. Washington, DC: U.S. Environmental Protection Agency. (PB 296797)

Palmer TY. 1976. Combustion sources of atmospheric chlorine. Nature 263: 44-46.

Pegg AE. 1977. Formation and metabolism of alkylated nucleosides: possible rule in carcinogenesis by nitroso compounds and alkylating agents. Advances in Cancer Research, 25:195-269.

Pohl LR, Bhooshan B, Whittaker NF, Krishna G. 1977. Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79:684-691.

Pohl LR, George JW, Martin JL, Krishna G. 1979. Deuterium isotope effect in in vivo bioactivation of chloroform to phosgene. Biochem. Pharmacol. 28:561-563.

Pohl LR, Krishna G. 1978. Deuterium isotope effect in bioactivation and hepatotoxicity of chloroform. Life Sci. 23:1067-1072.

Poirier LA, Stoner GD, Shimkin MB. 1975. Bioassay of alkyl halides and nucleotide base analogs by pulmonary tumor response in strain A mice. Cancer Res. 35: 1411-1415.

Putz VR, Setzer JV, Croxton JS, Phipps FC. 1979. Methyl chloride/drug interactions on performance. I. Effects of acute low level methyl chloride exposure combined with diazepam on behavioral and neurological measures of human activation levels. (Draft manuscript).

Rampy LW, Nitschke KD, Bell TJ, Burek JD. 1979. Interim results of two-year inhalation toxicologic studies of methylene chloride in rats and hamsters. Toxicol. Appl. Pharmacol. 48: A185.

Redford-Ellis M, Gowenlock AH. 1971a. Studies on the reaction of chloromethane with human blood. Acta Pharmacol. Toxicol 30: 36-48.

Redford-Ellis M, Gowenlock AH. 1971b. Studies on the reaction of chloromethane with preparations of liver, brain and kidney. Acta Pharmacol. Toxicol. 30: 49-58.

Repko JD, Jones PD, Garcia LS, Schneider EJ, Roseman E, Corum CR. 1977. Behavioral and neurologic effects of methyl chloride. Washington, DC: Department of Health, Education and Welfare. (DHEW Pub. NIOSH 77-125).

Reynolds ES, Yee AG. 1967. Liver parenchymal cell injury. V. Relationships between patterns of chloromethane-C^{14} incorporation into constituents of liver in vivo and cellular injury. Lab. Invest. 16:591-603.

Rosenkranz HS, Poirier LA. 1979. Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. J. Natl. Cancer Inst. 62:873-892.

Saita G. 1959. Prothrombin factors in acute poisoning by inhalation. Med. Lavoro 50:13-24. (Italian)

Scharnweber HC, Spears GN, Cowles SR. 1974. Chronic methyl chloride intoxication in six industrial workers. J. Occup. Med. 16: 112-113.

Schwarz F. 1926. Instances of poisoning and animal experimentation with methyl chloride. Dtsche. Z. Ges. Gerichtl. Med. 7:278-288. (German)

Schwetz BA, Leong BKJ, Gehring PJ. 1974. Embryo and feto-toxicity of inhaled chloroform in rats. Toxicol. Appl. Pharmacol. 28:442-451.

Sega GA, Cumming RB, Walton MF. 1974. Dosimetry studies on the ethylation of mouse sperm DNA after in vivo exposure to E [3/+] ethyl methanesulfonate. Mutat. Res. 24:317-333.

Sharp BB. 1930. Toxic effects of methyl chloride gas. Brit. Med. J. I: 336.

Simmon VS. 1978. In: Asher IM, Zervos C, eds. Structural correlates of carcinogenesis and mutagenesis--A guide to testing priorities. Washington, DC: Department of Health, Education and Welfare (DHEW Pub. FDA 78-1046), pp. 1-4, 163-171.

Singer B. 1975. The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. Prog. Nucl. Acid Res. Molec. Biol. 15: 219-284.

Singh HB, Salas LJ, Cabanagh LA. 1977. Distribution sources and sinks of atmospheric halogenated compounds. J. Air Pollut. Control Assoc. 27(4): 332-336.

Singh HB, Salas LJ, Shigeishi J, Scribner E. 1979. Atmospheric halocarbons, hydrocarbons, and sulfur hexafluoride: Global distributions, sources and sinks. Science 203: 899-903.

Sittig M. 1977. Pesticide process encyclopedia. Chemical technology review series. No. 81, pp. 76-78, 219-220, 335, 362.

Smith HH, Lotfy TA. 1954. Comparative effects of certain chemicals on Tradescantia chromosomes as observed at pollen tube mitosis. Amer J. Bot. 41: 589-593.

Smith WW. 1947. The acute and chronic toxicity of methyl chloride. III. Hematology and biochemical studies. J. Ind. Hyg. Toxicol. 29:185-189.

Smith WW, von Oettingen WF. 1947a. The acute and chronic toxicity of methyl chloride. I. Mortality resulting from exposures to methyl chloride in concentrations of 4000 to 300 parts per million. J. Ind. Hyg. Toxicol. 29: 47-52.

Smith WW, von Oettingen WF. 1947b. The acute and chronic toxicity of methyl chloride. II. Symptomatology of animals poisoned by methyl chloride. J. Ind. Hyg. Toxicol. 29: 123-128.

Soucek B. 1961. Retention and excretion of methyl chloride and carbon tetrachloride in rats. Arch. Gewerbepath. Gewerbehyg. 18:370-383. (German)

Sperling F, Macri FJ, von Oettingen WF. 1950. Distribution and excretion of intravenously administered methyl chloride. Arch. Ind. Hyg. Occup. Med. 1:215-224.

Spevak L, Nadj V, Felle D. 1976. Methyl chloride poisoning in four members of a family. Brit. J. Ind. Med. 33: 272-274.

Stanford Research Institute (SRI) (for NIOSH). Updated. Industrial hygiene assessment of selected chlorinated hydrocarbons project. Task 1 Report--methyl chloride.

SRI International (SRI) (for NIOSH). 1978a. Methyl chloride survey report of E.I. Dupont Corporation, Deepwater, New Jersey.

SRI International (SRI) (for NIOSH). 1978b. Methyl chloride survey report of Continental Oil Company, Conoco Chemicals, Westlake, Louisiana.

SRI International (SRI) (for NIOSH). 1978c. Methyl chloride survey report of General Electric Company, Waterford, New York.

SRI International (SRI) (for NIOSH). 1979a. Methyl chloride survey report of Conoco Chemicals, Westlake, Louisiana.

SRI International (SRI) (for NIOSH). 1979b. Methyl chloride survey report of the plant contact, Dow Corning Corporation, Midland, Michigan.

SRI International (SRI) (for EPA) (1979c). Atmospheric measurements of selected toxic organic chemicals. (Interim Report).

Stewart RD, Hake CL, Wu A, Graff SA, Forster HV, Keeler WH, Lebrun AJ, Newton PE, Soto RJ. 1977. Methyl chloride: Development of a biologic standard for the industrial worker by breath analysis. DHEW (NIOSH) MCOW-ENVM-MCM-77-1.

Sujbert L. 1967. Studies on the degradation of methyl chloride in mice. Arch. Toxicol. 22:233-235. (German)

Suntych F. 1956. Internal clinical picture of persons working with methyl chloride. Pracovni Lek. 8:91-95. (Czechoslovakian)

Suss R, Kinzel V, Scribner JD. 1973. Cancer: Experiments and concepts. New York: Springer-Verlag.

Theiss JC, Stoner GD, Shimkin MB, Weisberger EK. 1977. Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res. 37:2717-2720.

Thordarson O, Gudmundsson G, Bjarnason O, Johannesson T. 1965. Methyl chloride poisoning. Nordisk Med. 73:150-154. (Norwegian)

Tomatis L. 1979. The predictive value of rodent carcinogenicity tests in the evaluation of human risks. Ann. Rev. Pharmacol. Toxicol. 19:511-530.

Trosko JE, Chang CC. 1978. Relationship between mutagenesis and carcinogenesis. Photochem. Photobio. 28:157-168.

Trubecka G, Brzeski Z. 1968. Delirium syndrome in the course of methyl chloride intoxication. Med. Pracy 19:393-395. (Polish)

U.S. Department of Agriculture. 1962. Regulations for the enforcement of Federal Insecticide, Fungicide, and Rodenticide Act: interpretation with respect to warning, caution and antidote statements required to appear on labels of economic poisons. Federal Register, March 9, 1962, 27 FR 2267.

U.S. Environmental Protection Agency (EPA). 1973. Toxic pollutant effluent standards: List of toxic pollutants. Federal Register, September 7, 1973. 38 FR 24344.

U.S. Environmental Protection Agency (EPA). 1975. Preliminary economic assessment of possible regulatory action to control atmospheric emissions of selected halocarbons. Washington DC: U.S. Environmental Protection Agency (EPA-450/3-75-003).

U.S. Environmental Protection Agency (EPA). 1976. Chemical technology and economics in environmental perspectives. Task III--chlorofluorocarbon emission control in selected end-use applications. Washington, DC: U.S. Environmental Protection Agency (EPA-560/1-76-009).

U.S. Environmental Protection Agency (EPA). 1977. Investigation of selected potential environmental contaminants: Monohalomethanes. Washington, DC: U.S. Environmental Protection Agency (EPA-560/2-77-007).

U.S. Environmental Protection Agency (EPA). 1978a. Ethylene oxide. Rebuttable presumption against registration; maximum residue limits and daily levels of exposure. Federal Register, January 27, 1978, 43 FR 3800-3815.

U.S. Environmental Protection Agency (EPA). 1978b. Interim primary drinking water regulations. 43 FR 5756.

U.S. Environmental Protection Agency (EPA). 1979a. Short-term tests for carcinogens, mutagens and other genetoxic agents. Washington DC: U.S. Environmental Protection Agency (EPA 625/9-79-003).

U.S. Environmental Protection Agency (EPA). 1979b. Proposed health effects test standards for toxic substances control act test rules and proposed good laboratory practice standards for health effects. Federal Register, July 26, 1979, 44 FR 44054-44093.

U.S. Environmental Protection Agency (EPA). 1979c. Proposed health effects test standards for toxic substances control act test rules. Federal Register, May 9, 1979, 44 FR 27334-27375.

U.S. Environmental Protection Agency (EPA). 1979d. Unpublished data obtained from the U.S. Environmental Protection Agency Environmental Research Laboratory, Athens, Georgia, February 22, 1979.

U.S. Environmental Protection Agency (EPA). 1979e. Support document test data development standards: chronic health effects. Washington, DC: U.S. Environmental Protection Agency (EPA-560/11-79-001).

U.S. Food and Drug Administration (FDA). 1962. Food additives permitted in food for human consumption. Federal Register, May 14, 1962, 27 FR 4623.

U.S. Food and Drug Administration (FDA). 1970. FDA advisory committee on protocols for safety evaluations: panel on reproduction studies in the safety evaluation of food additives and pesticide residues. Toxicol. Appl. Pharmacol. 16: 264-296.

U.S. International Trade Commission (ITC). 1970-1975. Synthetic organic chemicals, United States production and sales. Washington, DC: U.S. International Trade Commission.

van Raalte HGS, van Velzen HGECT. 1945. Methyl chloride intoxication. Ind. Med. 14:707-709.

van Stee EW. 1976. Toxicology of inhalation anesthetics and metabolites. Ann. Rev. Pharmacol. Toxicol. 16: 67-79.

Villee C. 1971. Species differences in transport. Proceedings of a conference on toxicology: implications to teratology: 297-310.

von Oettingen WF. 1964. Halogenated hydrocarbons of industrial and toxicological importance. New York: Elsevier, pp. 5-25.

Vorhees CV, Brunner RL, Butcher RE. 1979a. Psychotropic drugs as behavioral teratogens. Science 205:1220-1225.

Vorhees CV, Brunner RL, Butcher RE, Sobotka TJ. 1979b. A developmental test battery for neurobehavioral toxicity in rats: a preliminary analysis using monosodium glutamata, calcium carrageenan and hydroxyurea. Toxicol. and Appl. Pharmacol. 50:267-282.

Walter B, Weiss A. 1951. Delayed results of acute methyl chloride intoxication. Med. Welt. 20:987-988. (German)

Weast RC, ed. 1978. CRC Handbook of Chemistry and Physics. 58th ed. Cleveland, OH: CRC Press.

Weinstein A. 1937. Methyl chloride (refrigerator) gas poisoning J. Am. Med. Assoc. 108: 1603-1605.

Weisburger J. 1978. A decision-point approach to carcinogen screening. In: Structural correlates of carcinogenesis and mutagenesis. A guide to testing priorities? Washington, DC: Department of Health, Education and Welfare (DHEW Pub. FDA 78-1046) pp. 45-54.

White JL, Somers PP. 1931. The toxicity of methyl chloride for laboratory animals. J. Ind. Hyg. Toxicol. 13: 273-275.

Wiernikowski A, Korohoda J, Guzik E, Wojdyla Z. 1974. Collective intoxication with methyl chloride. Med. Pracy 25: 571-573. (Czechoslovakian)

Wilson JG, Fraser FC. 1977. Handbook of Teratology. Vol. 1. New York: Plenum Press.

Windholz M, ed. 1976. The Merck index, 9th ed. Rahway, NJ: Merck and Co., Inc.

Wood MWW. 1951. Cirrhosis of the liver in a refrigeration engineer attributed to methyl chloride. Lancet I: 508-509.

World Health Organization (WHO). 1967. Principles for testing of drugs for teratogenicity. WHO Tech. Rep. Ser. No. 364. Geneva: World Health Organization.

Yevtushenko GY. 1966. The toxicology of methyl chloride. Gig. Tr. Prof. Zabol. 10:20-25. (Russian)

Yevtushenko GY. 1967. Metabolism of methyl chloride. Farmakol. Toksikol. 30:239-240. (Russian)