

# federal register

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**ENVIRONMENTAL PROTECTION  
AGENCY**

**40 CFR Part 799**

**[OPTS-470020; TSH-FRL 2810-7]**

**Chloromethane; Withdrawal of  
Proposed Health Effects Test Rule**

**AGENCY:** Environmental Protection  
Agency (EPA).

**ACTION:** Proposed rule: withdrawal.

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**SUMMARY:** This notice presents EPA's  
final decision not to require  
oncogenicity and structural  
teratogenicity testing of chloromethane

(CAS No. 74-87-3) and to withdraw these proposed testing requirements for this chemical. The notice also discusses available information on chloromethane's reproductive and mutagenic potential, and provides reasons for EPA's not initiating test rules for these effects.

**FOR FURTHER INFORMATION CONTACT:**

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**SUPPLEMENTARY INFORMATION:** EPA is withdrawing its proposed rule requiring oncogenicity and structural teratogenicity testing of chloromethane.

**I. Background**

Section 4(a) of the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2006 *et seq.*, 15 U.S.C. 2603 *et seq.*) authorizes the Administrator of EPA to promulgate rules which require manufacturers and processors to test chemical substances and mixtures. Data developed through these programs are used by EPA in assessing the risks the chemicals may present to human health and the environment.

Section 4(e) of TSCA established an Interagency Testing Committee (ITC) to recommend a list of chemicals for EPA to consider for promulgation of testing rules under section 4(a) of the Act. The ITC designated chloromethane for priority consideration in its initial report, published in the *Federal Register* of October 12, 1977 (42 FR 55026). The ITC recommended that chloromethane be tested for carcinogenicity, mutagenicity, teratogenicity and other chronic effects with specific emphasis on the central nervous system, liver, kidneys, bone marrow and cardiovascular system. The ITC recommendations were based upon high domestic production levels (350 million pounds reported in 1974), significant release of 15 million pounds per year to the environment, high numbers of exposed workers (estimated 31,000), structural similarity to other reported carcinogens, implication in chronic diseases and mutagenic activity in micro-organisms.

In the *Federal Register* of July 18, 1980 (45 FR 48524), the Agency issued a proposed rule requiring the manufacturers and processors of chloromethane to conduct oncogenic and structural teratogenic effects testing because available data indicated that chloromethane may present an unreasonable risk of injury to human

health. A requirement for oncogenicity testing was proposed by EPA because chloromethane had been shown to be mutagenic in bacteria and capable of causing chromosomal aberrations in plants. It was also known to be a direct alkylating agent in human and animal tissues, to be structurally related to other halomethanes thought to have oncogenic potential, and to be metabolized to formaldehyde which appeared to be oncogenic in some studies. The requirement for teratogenicity testing was based on chloromethane's lipid solubility, low molecular weight, probability of crossing the placenta, and association with a documented fetal death. The proposed rule noted that chronic toxicity/oncogenicity and teratogenicity testing was being sponsored by the Methyl Chloride Industry Association (MCIA). The chronic toxicity/oncogenicity study was being conducted by Battelle Laboratories under contract to the Chemical Industry Institute of Toxicology (CIIT); teratology testing was to be done at CIIT. The proposed rule also noted that after reviewing both test protocols and interim test data from the chronic toxicity/oncogenicity study, EPA found certain limitations that led the Agency to question the abilities of these studies to adequately detect any oncogenic or teratogenic hazard that chloromethane might pose to humans.

After publishing its proposed test rule for chloromethane, EPA received written and oral comments on its proposal. As a result of comments received from MCIA, the Agency decided to await receipt of the study reports from the industry testing program before making its final testing decision (Ref. 1).

CIIT and MCIA have provided the Agency with draft and final reports of their 24-month chronic toxicity/oncogenicity study and teratogenicity study for chloromethane. In addition, CIIT and MCIA have submitted a reproductive effects study in rats for chloromethane.

**II. Chemical Profile**

The chemical chloromethane (methyl chloride, CAS No. 74-87-3) is produced in the vapor phase by reacting methanol with hydrochloric acid and a catalyst such as alumina. Chloromethane production in the United States is expected to remain steady or to slowly increase over the next few years from an estimated figure of greater than 600 million pounds for 1982. Estimates indicate that 75 to 80 percent of the chloromethane produced today is consumed in the production of methyl silicon compounds and tetramethyl lead. Other primary uses include the

manufacture of pesticides, quaternary amines, methylated compounds, and various chlorinated methanes. Minor uses include solvent, industrial refrigerant, and blowing agent. Exports constitute approximately 5 percent of production.

For the period of 1972-1974, the National Institute for Occupational Safety and Health (NIOSH) estimated the maximum number of U.S. workers exposed to chloromethane to be approximately 50,000 people at nearly 4,000 plants or facilities (Ref. 2). Recent information from an engineering analysis conducted by EPA during 1983 show that 1,500 to 2,000 workers are exposed to chloromethane at no more than 50 plants or facilities (Ref. 3). This analysis also found that in all usage areas for which data are available, chloromethane exposure levels are generally within the 100 part per million (ppm) 8-hour time-weighted average (TWA) established by the Occupational Safety and Health Administration (OSHA). Excursions above this limit do occur but are intermittent and are believed to be brief.

Besides the 100 ppm TWA, OSHA has also established a 200 ppm 15-minute chloromethane ceiling concentration and a maximum acceptable peak concentration allowance of 300 ppm for 5 minutes over a 3-hour period (Ref. 2). The American Conference of Governmental Industrial Hygienists (ACGIH) recommended the establishment of a threshold limit value (TLV) of 100 ppm in 1971, but reduced their recommendation to 50 ppm in 1982 (Ref. 2). The ACGIH also recommends a 15-minute exposure limit of 125 ppm to occur no more than four times a day.

**III. Toxicity Profile**

The following discussions present new health effects test data on chloromethane. After reviewing and evaluating this testing and the test results, EPA has decided to withdraw each of the proposed health effect testing requirements for chloromethane.

**A. Development Toxicity**

In its proposed test rule, EPA required that structural teratogenic effects testing of chloromethane be conducted. However, after publication of the proposed rule, teratogenicity testing of chloromethane was initiated at CIIT under the sponsorship of the MCIA (Ref. 4). In these studies pregnant Fischer-344 rats and C57BL/6 mice were exposed from gestation day (gd) 7 to gd 20, and from gd 6 to gd 18, respectively, for 6 hours daily to atmospheres containing 0, 100, 500 or 1,500 ppm chloromethane. On

the final day of gestation all animals were sacrificed for evaluation of maternal reproductive and fetal parameters.

Upon review of the structural teratogenicity study report for chloromethane, EPA found that the data from the rat study indicated that there were no chloromethane-induced external, skeletal, or visceral malformations in the fetuses. In fetuses from the highest exposure group (1,500 ppm), retardation in ossification was observed. Trend analysis indicated that the fraction of fetuses per litter with retarded ossifications may have been increased in the 500 ppm group as well as the 1,500 ppm group. Maternal food consumption and body weight were depressed in dams exposed to 1,500 ppm when measured on gd 15 and 20. Weight gain was depressed in dams exposed to either 500 or 1,500 ppm chloromethane during the first week of exposure (gd 7-15). For the 1,500 ppm group body weight at sacrifice was also depressed as was body weight minus gravid uterine weight. No other maternal or reproductive parameters were affected in any of the exposure groups. Because of these findings, the Agency has concluded that exposure to chloromethane for pregnant rats, during critical periods of embryo and fetal development, was not teratogenic at concentrations which elicited maternal and fetal toxicity.

Results for the mouse study showed that chloromethane was severely toxic to pregnant C57BL/6 mice carrying B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> fetuses following at least 4 days of exposure to 1,500 ppm. These dams exhibited urogenital bleeding and central nervous system (CNS) dysfunction beginning on the fourth day of exposure, i.e., gestation day 10. Termination of exposures and histopathological examination of the dams revealed a CNS lesion specific to the internal granule cell layer of the cerebellum.

On gd 18, the females from the lower treatment groups, all of which survived, were sacrificed for evaluation of maternal reproductive and fetal parameters, with 24 females pregnant in the 0 ppm group, 20 in the 100 ppm group, and 17 in the 500 ppm group. In these dams, no alterations were seen in body weight or weight gain during the exposure period. Intake of food and water was elevated relative to controls in the 500 ppm group during gd 6-14. Maternal reproductive parameters were not affected in the 500 or 100 ppm groups relative to controls.

Live male fetuses in the 100 ppm group exhibited an increase in crown-rump length relative to controls. There

were no other alterations in external appearance in fetuses from any of this exposure groups. Fifty percent of each litter was examined for visceral defects, 50 percent for skeletal defects. Visceral examination of mouse fetuses revealed a small incidence of heart defects in litters of the 500 ppm group. The anomaly, a reduction or absence of the atrioventricular valve, chordae tendineae, and papillary muscle, was observed on the left side (bicuspid valve) in three fetuses and right side (tricuspid valve) in six fetuses. Three males and six females were affected. No single fetus had both sides involved; one litter had fetuses with left and right side involvement, and five of the six affected litters also had fetuses with normal hearts. In selected skeletal districts, the degree of ossification was positively correlated with increasing exposure concentrations. However, this observation in this particular study may not be related to chemical exposure. No embryo-fetal toxicity or teratogenicity was associated with exposure of mice to 100 ppm of chloromethane during critical periods of embryo and fetal development.

To confirm the teratogenic effects observed in the original mouse study and to attempt to establish a dose-response relationship for those effects, CIIT determined that a supplemental or follow-up teratology study on mice should be conducted. In the follow-up study, pregnant C57BL/6 female mice were exposed daily for 6 hours to atmospheres containing 0, 250, 500, or 750 ppm chloromethane, from gd 6 to gd 18 (Ref. 4). Females exposed to 750 ppm chloromethane exhibited ataxia commencing on the seventh day of exposure (gd 12). These dams also showed hypersensitivity to touch or sound, tremors and convulsions. Six females in the 750 ppm group died and one was sacrificed *in extremis* prior to scheduled sacrifice. On gd 18, all other females were sacrificed for evaluation. Only dams exposed to 750 ppm exhibited a decrease in body weight by gd 18, weight gain during the gestation period and absolute weight gain (weight gain minus gravid uterine weight) versus controls. There were no treatment-related effects on these parameters in the lower exposure groups. None of the groups exhibited exposure-related differences in pregnancy rate or maternal liver weight. In addition, there were no significant treatment-related effects on number or percentages of implantations, resorptions, incidence of dead fetuses or non-live (dead plus resorbed) fetuses per litter, nor on the number of live fetuses per litter, sex-ratio or mean fetal body weight per litter

in any of the treatment groups relative to controls.

There was an exposure-related increase across groups in the number and percentage of affected (non-live plus malformed) fetuses per litter with the incidence of affected fetuses in the 750 ppm group higher than controls. Visceral examination of the thoracic cavity of all of the fetuses revealed an increase in the incidence of heart defects in the 500 and 750 ppm groups relative to controls. There was an exposure-related increase for the following parameters: numbers and percentage of malformed fetuses and malformed male and female fetuses per litter, with the incidence of all these parameters in the 750 ppm group significantly higher than controls. Numbers of litters with malformations, and numbers of litters with malformed males and females were all elevated in the 750 ppm group versus controls. Numbers of malformed fetuses were elevated in the 500 ppm and 750 ppm, but not in the 250 ppm group relative to the controls. In total, 38 fetuses were malformed, 37 with heart defects.

The Agency has determined from its review of these data and the final study report submitted by CIIT that sufficient test data to reasonably predict the extent of the potential developmental toxicity and teratogenic hazard to humans from exposure to chloromethane now are available. The Agency also concludes that, because positive results were found in this testing, the limitations originally cited by EPA in the study design are no longer of concern. Therefore, EPA believes that the statutory findings necessary to require developmental toxicity or structural teratogenicity testing under section 4(a) of TSCA for chloromethane cannot be made at this time, and is withdrawing its proposed rule of July 18, 1980, requiring structural teratogenicity testing of chloromethane.

#### B. Reproductive Effects

As part of the ITC's teratogenicity testing recommendation, the committee also called for the initiation of studies to determine the extent of the potential hazard of chloromethane to the reproductive system and the fetus. In its response to the ITC, EPA noted that there were no data to support a conclusion that chloromethane may present an unreasonable risk of reproductive effects. Consequently no testing was proposed. However, recent data from CIIT indicated that chloromethane causes reproductive effects. These effects were first noted in CIIT's chronic toxicity/oncogenicity

inhalation study, which is discussed in detail in Unit III.C., and demonstrated that chloromethane elicits testicular effects in male Fisher-344 rats.

Considering the testicular effects data (i.e., epididymal sperm granulomas and degeneration of the testicular germinal epithelium) and the results of the teratology studies, CIIT undertook a study to evaluate the effects of chloromethane on reproduction and fertility in the rat (Ref. 5). This study, now completed, is adequate for assessment purposes.

In this study, male and female Fisher-344 rats were exposed to chloromethane by inhalation (0, 150, 475 or 1,500 ppm, 6 hours/day, 5 days/week, 40 males and 80 females per group). The only treatment-related clinical signs were a 10 to 20 percent body weight gain depression (BWGD) in both males and females exposed to 1,500 ppm after 2 weeks of exposure and a 5-7 percent BWGD in 475 ppm exposed animals after day 57. After 10 weeks the exposure schedule was changed to 6 hours/day, 7 days/week and each male was mated to two exposed females. The mating period lasted 2 weeks and then 10 males/group were necropsied. The only treatment-related lesions found were severe testicular degeneration (10/10) and granulomas in the epididymis (3/10) in the 1,500 ppm males. The remaining 30 males per group were then mated during a 2-week period with 60 unexposed females. The exposed females were continued on exposure from the start of mating to day 18 of gestation (6 hours/day, 7 days/week). The females were not exposed from gd 18 to postnatal day 4, but exposure (6 hours/day, 7 days/week) of these females was resumed from postnatal day 4 to postnatal day 28. There were no differences between groups in the number of exposed or unexposed females that mated as evidenced by copulation plugs. No litters were born to exposed or unexposed females mated to the 1,500 ppm males. There was no significant difference in the number of litters produced by the 150 ppm groups when compared to the control groups. Fewer litters were born in the 475 ppm groups than in the control groups. No differences in litter size, sex ratio, pup viability or pup growth were found among the 475 ppm, 150 ppm or control  $F_0$  groups. When bred 10 weeks after the cessation of exposures, 5 of 20 of the 1,500 ppm  $F_0$  males had regained the ability to sire normal litters. The same number of 475 ppm  $F_0$  males were proven fertile as control  $F_0$  males. After weaning,  $F_1$  pups from the 475, 150 and 0 ppm groups were exposed to the same

concentrations of chloromethane for 10 weeks and then mated. A trend toward decreased fertility was found in the 475 ppm group.

The Agency has concluded from its review of these data and the final study report submitted by CIIT that sufficient test data to reasonably determine or predict the risk of reproductive effects in humans exposed to chloromethane now are available.

#### C. Chronic Toxicity and Oncogenicity

In its proposed test rule, EPA also planned to require that oncogenicity testing of chloromethane be conducted. However, prior to publication of the proposed rule, a 2-year chronic toxicity/ oncogenicity study had been initiated under the sponsorship of the MCLA and conducted by Battelle Laboratories under contract to CIIT. In this study, male and female Fischer-344 rats and  $B_6C_3F_1$  mice were exposed by inhalation to target concentrations of 50, 225, or 1,000 ppm chloromethane for 6 hours/ days, 5 days/week. The final chronic toxicity/ oncogenicity test report indicates that the Fischer-344 rat's life expectancy was not affected by exposures to chloromethane, whereas both male and female mice were adversely affected at the 1,000-ppm dose level (Ref. 6).

Nearly all male and female mice examined prior to sacrifice from the 1,000-ppm exposure group at 18, 21 (male), or 22 (female) months had signs of neurofunctional impairment (clutch response) that were different from control animals. No exposure-related effect was observed in animals from the lower exposure groups. In male and female rats no neurofunctional impairments were reported that are attributable to chloromethane exposure.

The growth of male mice through the first 18 weeks at 1,000-ppm concentration was less than that of the control mice. The rate of growth for male and female at 1,000 ppm and female rats at 225 ppm of chloromethane was also reduced during the first 24 weeks.

Compound-related hepatocellular changes were observed at the 6-month sacrifice in mice from the 1,000-ppm group. These changes, which included centrilobular to midzonal hepatocellular vacuolization, karyomegaly, cytomegaly, multinucleated hepatocytes, and degeneration, were seen only in males until the 18 to 22-month period when females developed similar but less severe lesions.

Renal tubuloepithelial hyperplasia and karyomegaly were seen at 12 months in 1,000-ppm male mice and progressed in severity and prevalence

throughout the study. An increase in renal tumors was noted in 1,000-ppm male mice sacrificed or dying between 12 and 21 months, including renal cortical adenoma, renal cortical adenocarcinoma, papillary cystadenoma, papillary cystadenocarcinoma, and tubular cystadenoma. Sixteen of seventy-seven animals in the highest dose group were demonstrated to have renal adenomas and adenocarcinomas. A renal cortical adenoma was demonstrated in two 225-ppm male mice at the 24-month terminal sacrifice.

Renal cortical cysts were predominantly seen in mice in the 1,000-ppm group, whereas microcysts were noted most frequently in the 50-ppm group at 24 months. Both occurrences were different from controls and may be related to chloromethane exposure.

Cerebellar lesions first appeared in male and female mice at the 18-month sacrifice from the 1,000-ppm group. The lesion, which was characterized by degeneration and atrophy of the cerebellar granular layer, did not appear in mice from any other exposure group or in the controls. This lesion is considered to be related to chloromethane exposure.

Splenic alterations, ranging from lymphoid depletion to splenic atrophy, were present in male and female mice from the 1,000-ppm group as early as 6 months and progressed throughout the study. Depletion was noted in only one control mouse during the study at the month sacrifice. Splenic atrophy was noted in mice dying spontaneously between 0 and 17 months, but was not apparently increased over controls until the 18 to 24-month period. Both lesions are considered by EPA and CIIT to be related to chloromethane exposure.

In rats, the testes were the only organs considered to have chloromethane-induced lesions. Bilateral, diffuse degeneration and atrophy of the seminiferous tubules of the testes were first noted in a 1,000-ppm male rat at the 6-month sacrifice. The occurrence of this lesion increased through the 18-month sacrifice. By 24 months, all male rats had interstitial cell hyperplasia or adenomas associated with aging, and it was impossible to detect further the exposure-related seminiferous tubular degeneration and atrophy. However, with increasing exposure concentrations, the resultant decrease in bilateral compressive degeneration and atrophy and the increase in unilateral compressive degeneration and atrophy (cause by testicular tumors) correlated with a decrease in interstitial cell tumor size.

This observation was supported by decreased testicular weights and tests/body weight ratios in rats exposed to 1,000 ppm of chloromethane.

In the Agency's review of this study, particularly the mouse data, the maximum tolerated dose (MTD) appears to have been exceeded as indicated by early deaths in the 1,000 ppm dose group. Furthermore, control male mice had poor survival, with only 17 percent surviving to terminal sacrifice. In the male mouse study, therefore, it is difficult to compare lesions or neoplasms in the treated groups with the respective control group because survival in the control group was poor. In the female mouse study survival in the controls was good, but no neoplasms are reported.

The rat study suffers in that it appears that higher doses could have been administered; though how much higher is uncertain. While some effect on weight gain was seen in males and females exposed to 1,000 ppm of chloromethane, presumably an MTD for these rats, no effect on survival or clinical parameters was seen.

In conclusion, the Agency believes that a good attempt was made by CIIT to conduct a proper chronic toxicity/ oncogenicity study with chloromethane by the inhalation route. The chemical used was of high purity (99.97 percent). The animals used by CIIT (Fischer 344 rats and B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice of both sexes) are routinely used by the National Toxicology Program in long-term studies. The historical incidence of neoplastic and non-neoplastic lesions in these rodents has been well characterized. Animal husbandry prior to the initiation of the study appears adequate (i.e. quarantine); and a subchronic study was conducted to estimate the dosages to be used in the chronic study. Three doses (30 ppm, 250 ppm, and 1,000 ppm) were employed in the study for both species, although only the 1,000 ppm dosage should be considered sufficient for an oncogenicity study. Sufficient numbers of animals were used (approximately 120 per group), with serial sacrifices and complete clinical chemistry, hematology, and urinalysis performed at selected intervals to indicate the health of the animals and target organ toxicities. With 25 to 40 animals programmed to be killed from each group by the eighteenth month of the study, adequate survival was anticipated at terminal sacrifice, barring any unforeseen infection in the animal colony or high mortality due to chemical-induced, life-shortening lesions.

However, some problems arose during the study in the area of animal

husbandry, namely, missexing and pregnancies. Two additional problems occurred in the handling of the inhalation chambers: (1) Mix-up in dosing on three consecutive days, and (2) brief exposure of control animals to chloromethane. While these problems are serious flaws in the conduct of the experiment, the Agency believes they do not sufficiently compromise the experiment to negate its results. The effect of pregnancy on the handling of chloromethane by female mice is not known (the report does not mention if missexing occurred also in control mice). Exposure of low dose mice (50 ppm) to a high dose of chloromethane (1,000 ppm) early in the study may have a slight effect (increase) on the incidence of tumors in this group. The exposure of high dose mice (1,000 ppm) to low doses of chloromethane (50 ppm) for 3 days at the beginning of the study, probably would not have a significant effect on the incidence of neoplasms in this group. Brief exposure of control animals to chloromethane may have a slight effect on the incidence of neoplasms in this group. However, taken in the light of a 2-year test, the effect, most likely, would be minimal.

The Agency has determined that the study results are sufficient to indicate that chloromethane is a possible human carcinogen. The Agency also concludes that the technical problems with the study do not negate the positive findings of the test.

#### *D. Metabolism Test Data on Chloromethane*

As new health effect information became available under its initial health effects testing plan for chloromethane, the MClA and CIIT decided to conduct further testing to clarify studies (Ref. 7). Short-term studies chosen by CIIT and MClA to elucidate chloromethane's mechanism of toxicity showed that the disposition of <sup>14</sup>C-chloromethane in male Fischer-344 rats and B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice after a single 6-hour inhalation exposure to 100, 375 or 1,500 ppm resulted in 8 to 21 percent of the radioactivity remaining in tissues and carcasses of the animals 48 hours after exposure. This study also showed that chloromethane was extensively metabolized to carbon dioxide in both species, accounting for about 40 to 60 percent of the total body burden of radioactivity.

In a subsequent study to establish a metabolic pathway for generating the labelled carbon dioxide, exposure of rats for 6 hours to 500 or 1,500 ppm chloromethane was found to markedly decrease the reduced glutathione (GSH) in liver, lung, and kidney tissues. GSH blood levels were not affected by

exposure to the chemical. This observation suggested to CIIT that chloromethane did not spontaneously methylate cellular sulfhydryl groups *in vivo*, but rather required catalysis by one of more enzymes not present in blood. Subsequent experiments implicated a GSH-S-alkyltransferase as the enzyme probably catalyzing the conjugation reaction. Further experimentation was interpreted by CIIT to show that macromolecular fractions, such as nucleic acids and proteins, isolated from rats exposed to <sup>14</sup>C-chloromethane contained significant amounts of nonextractable radioactivity. Chromatographic analyses of the DNA indicated that the radioactivity was incorporated into normal purine bases, and did not represent any detectable methylation of the bases.

*In vitro* DNA alkylation studies conducted by CIIT using non-radioactive chloromethane and high-pressure liquid chromatography suggested that chloromethane was a weak alkylating agent. These data led CIIT to suspect that an epigenetic mechanism may be responsible for the increased incidence of renal tumors in male mice chronically exposed to chloromethane.

Further study was undertaken to evaluate the nature of the radioactivity associated with the protein fraction using rats treated with cycloheximide, a protein synthesis inhibitor. This study showed that radioactivity associated with the protein fraction was dramatically decreased, suggesting that a majority of the radioactivity was present as a result of its incorporation into amino acids prior to protein synthesis.

A possible mechanism by which chloromethane might be incorporated into macromolecules was thought, by CIIT, to occur through an intermediate capable of entering a one-carbon biosynthetic pathway. This was confirmed by further CIIT testing in which pretreatment with an inhibitor of tetrahydrofolate (an enzyme cofactor involved in one-carbon transfer reactions) significantly reduced radioactivity associated with tissue macromolecules and increased formate concentrations in urine and blood resulting from exposure to <sup>14</sup>C-chloromethane. Further metabolism studies showed that formaldehyde appeared to be a probable intermediate in the metabolism of chloromethane.

In another series of tests, CIIT demonstrated that inhalation of chloromethane by mice resulted in a concentration-dependent depletion of glutathione in liver, kidney, and brain

tissues. Exposure for 6 hours to 100 ppm lowered liver GSH by over 40 percent, while exposure to 2,500 ppm reduced GSH to 2 percent of control levels. For those exposures which decreased liver GSH to less than 20 percent of control levels, the extent of liver GSH depletion was correlated with the capacity of a liver fraction to undergo spontaneous lipid peroxidation. Addition of GSH to the incubation prevented lipid peroxidation. These findings suggested that depletion of GSH by chloromethane to levels insufficient to prevent the occurrence of spontaneous lipid peroxidation may underlie the hepatotoxicity of the chemical.

From the new information on the biochemical mechanisms of chloromethane metabolism, CIIT developed a study program designed to further show the relationship of toxicity to metabolism and in March 1982 made its proposed plan available to the Agency. The primary objective of this project was to evaluate the role of metabolism of chloromethane, in particular that of the GSH-chloromethane conjugate, in mediating the acute and chronic toxicity of chloromethane. Included within this objective was an evaluation of the mechanism(s) underlying the acute and/or subacute brain, liver, and kidney toxicity of chloromethane, and the renal oncogenicity observed with chronic exposures of male mice to chloromethane. CIIT believed the correlation between target sites in the acute toxicity studies and those in the chronic studies suggested that the mechanism of toxicity in short-term exposures could be extrapolated to chronic exposures. As a result of this correlation CIIT updated its study program (Ref. 8) to investigate the following specific areas: Comparison of the absorption, metabolism, and excretion of chloromethane by male and female mice; development of biochemical markers to evaluate the acute toxicity of chloromethane; determination of the relationship and relevance of the proposed metabolic pathway to the acute toxicity of chloromethane; evaluation of the role of formaldehyde as an intermediate in chloromethane toxicity; investigation of the genotoxic potential of chloromethane; and, characterization of the histopathology of the testicular lesions induced by chloromethane in rats.

As this testing program is completed and new data become available, the Agency believes the study results may further define the biochemical

mechanism through which chloromethane induces oncogenicity.

#### E. Mutagenicity

In its initial report to the Agency, the ITC also recommended supplemental mutagenicity testing consisting of chromosomal aberration studies. The ITC based this recommendation on positive *Salmonella* mutagenicity results in a microsomally activated test system. In the July 18, 1980 proposed rule, EPA stated that mutagenic risk from exposure to chloromethane can most reasonably be determined by performing a sequence of tests for both gene mutation and chromosomal aberration. EPA, however, deferred proposing mutagenicity testing via tiered testing because the Agency had not at the time it issued the proposed test rule yet developed specific criteria for sequencing decisions nor a standard approach to be applied for DNA alkylation tests in tiered gene mutation studies. However, because of existing information showing chloromethane's ability to cause direct-acting gene mutations in bacteria and chromatid breakage in the pollen grains of *Tradescantia paludosa*, and in the interest of proceeding with the characterization of chloromethane's mutagenic potential, EPA decided to sponsor all studies in a proposed mutagenicity sequence (45 FR 48540; July 18, 1980) except the final tiered tests.

From these Agency-sponsored studies, test data show that following acute inhalation of chloromethane (6 hours/day for 5 days), dominant lethal effects (chromosomal aberrations) occurred in rats (Ref. 9). Animals exposed to 2,000 or 3,000 ppm (target doses) showed a dominant lethal effect on the postmeiotic germ cells, and the effect was more pronounced in animals exposed to 3,000 ppm. The effect was described as transient, since no dominant lethal effects were seen in animals mated during the week following final exposure. The *Drosophila* sex-linked recessive lethal gene mutation assay results were also positive and confirm other studies which indicate that chloromethane induces gene mutations (Ref. 10).

Under the tiered mutagenicity testing scheme for gene mutation testing now employed by EPA in test rules, the positive findings in the Agency-sponsored *Drosophila* sex-linked recessive lethal assay would trigger a mouse specific locus study. The positive findings in the Agency-sponsored dominant lethal assay would trigger heritable translocation testing to determine not only the mutagenic

activity of chloromethane but also the heritability of such effects.

Nevertheless EPA has determined that in this instance the available data, though inadequate to quantify the mutagenic risks chloromethane poses to humans, are sufficient to qualitatively classify chloromethane as a potential human germ-cell mutagen. Consequently the Agency is not requiring further chromosomal aberration or gene mutation testing at this time.

#### IV. Decision to Withdraw Proposed Testing Requirements

By Considering the available oncogenicity, reproductive effects, mutagenicity, and metabolism data for chloromethane and its workplace exposure profile, EPA believes there is sufficient information and experience to reasonably determine or predict chloromethane's potential to cause health effects on humans.

Therefore, the Agency finds that sufficient data and experience are available pursuant to section 4(a) of TSCA to reasonably determine or predict the health effects of chloromethane, and that no further testing is required at this time. Therefore, EPA is withdrawing all health effects testing requirements for chloromethane as proposed in the Federal Register of July 18, 1980 (45 FR 48524).

The test rule was proposed in 40 CFR Part 773 and subsequently recodified 40 CFR Part 799 (49 FR 39820, October 10, 1984).

#### V. Public Record

The record, containing the basic information considered by the Agency in developing its decision, is available for inspection in the OPTS Reading Room from 8 a.m. to 4 p.m. Monday through Friday, except legal holidays, in Rm. E-107, 401 M Street SW., Washington, D.C. 20460. The Agency will supplement this record periodically with additional relevant information received.

The EPA has established a public record of this testing decision (docket number OPTS-47002D). This record includes:

##### A. Supporting Documentation

(1) The Federal Register notice designating chloromethane to the priority list (42 FR 55026; October 12, 1977) and comments received in response thereto.

(2) The Federal Register notice proposing health effects testing requirements for chloromethane (45 FR 48524, July 18, 1980) accompanying

supporting documents, and comments received in response thereto.

(3) Communications consisting of letters, contact reports of telephone conversations, and meeting summaries.

(4) Testing program being sponsored by MClA and its supporting documents.

#### *B. References*

(1) EPA. (U.S. Environmental Protection Agency). Summary of TSCA Section 4(a) Public Meeting on Chloromethane. Washington, D.C. October 30, 1980.

(2) NIOSH. National Institute for Occupational Safety and Health. Computer printout: National Occupational Hazard Survey, 1972-1974. Retrieved December 5, 1978. Washington, D.C.

(3) Preregulatory Assessment of Industrial Methyl Chloride Exposure. Prepared by PEDCo Environmental, Inc. for Office of Pesticides and Toxic Substances, EPA. EPA Contract No. 68-02-3935. January 1984.

(4) CIIT. (Chemical Industry Institute of Toxicology). Study reports for

chloromethane. Letter from A.E. McCarthy (CIIT) to S. Newburg-Rinn, Office of Pesticides and Toxic Substances, EPA. December 23, 1981.

(5) CIIT. Reproduction in F-344 rats exposed to methyl chloride by inhalation for two generations. Final report by CIIT. CIIT, RTP, N.C. April 13, 1984.

(6) Battelle Columbus Laboratories. A chronic inhalation toxicology study in rats and mice exposed to methyl chloride. Four volume final report submitted to CIIT. December 31, 1981.

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(8) CIIT. Additional documents relating to proposed rule (80-T-126). Letter from J.E. Gibson (CIIT) to Document Control Officer, Office of Pesticides and Toxic Substances, EPA. March 18, 1982.

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of Toxic Substances, EPA. EPA Contract No. 68-01-5079. August 1984.

(10) Bioassay Systems Corporation. Drosophila Sex Linked Recessive Lethal Test on Chloromethane. Final Report. Prepared by Ruby Valencia, Univ. of Wisc., Madison, WI. Subcontract No. 416-81 of BSC 10506.

(Sec. 4. TSCA (Pub. L. 94-469, 90 Stat. 2006; 15 U.S.C. 2603))

#### **List of Subjects in 40 CFR Part 799**

Environmental Protection Agency (Testing), Environmental Protection, Hazardous material, Chemicals, Testing.

Therefore, 40 CFR 799.130 originally proposed at 45 FR 48524, July 18, 1980 and subsequently recodified at 49 FR 39820, October 10, 1984 is withdrawn.

Dated: April 23, 1985.

J.A. Moore,

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[FR Doc. 85-10916 Filed 5-6-85; 8:45 am]

BILLING CODE 6560-50-M