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Project Summary

Health Assessment Document for Chloroform

The Office of Health and Environmental Assessment of the Office of Research and Development, Environmental Protection Agency (EPA), has prepared this health assessment to serve as a "source document" for EPA use. While the assessment was originally initiated for use in evaluating chloroform as a toxic air pollutant under provisions of Section 112 of the Clean Air Act, the scope of the assessment covers other exposure pathways such that it is useful to other media-specific programs in the EPA.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and conclusions have been prepared in order to qualitatively and quantitatively identify the toxicity of chloroform. Toxic effect exposure levels and other measures of dose-response are discussed, where appropriate, to place the nature of the health responses in perspective with chloroform levels in the environment.

Information regarding sources, of chloroform release to the environment, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the environment. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

This Project Summary was developed by EPA's Environmental Criteria and Assessment Office, Research Triangle Park, NC, to highlight the key findings of the health assessment document (see Project Report ordering information at back).

Introduction

Chloroform ($CHCl_3$) is a colorless, volatile, nonflammable, liquid used primarily in the production of chlorodifluoromethane (90%) and for export (5%). Nonconsumptive uses (5%) include use as a solvent, as a cleaning agent, and as a fumigant ingredient. Although chloroform production and capacity have declined recently, 1981 data place direct production of chloroform in the United States at 184 million kg, with indirect production estimated at 13.2 million kg. Also, based on 1981 data, the amount of chloroform in the U.S. emitted to air is estimated to be 7.2 million kg, with discharges to water of 2.6 million kg, and discharges on the land of 0.6 million kg.

Chloroform is ubiquitous in the environment, having been found in urban and non-urban locations. It has a characteristic odor and is detectable at about 200 ppm. There have been reports of a northern hemisphere background average of 14 ppt (10^{-12}v/v), with an average in the southern hemisphere of <5 ppt, and a global average of 8 ppt. However, a more recent report suggests the ratio of hemispheric concentrations (north vs. south) may be less dramatic, more on the order of 1.6. For the most part, urban ambient air concentrations remain Ω1000 ppt, and rural or remote locations can be <10 ppt. There are some notable exceptions, however, but the reasons for them are not readily apparent. The highest values reported were in Rutherford, New Jersey (31,000 ppt), and Niagara Falls, New York (21,611 ppt).

Physical and Chemical Properties, and Analysis

Hydroxyl radical oxidation is the primary atmospheric reaction of chloroform. Based on the rate constant for reaction with chloroform, a half-life of 11.5 weeks is expected. The principal products from this reaction are HCl and CO_2. It has been estimated that roughly 1% of the tropospheric chloroform will diffuse into the stratosphere, based on a lifetime of 0.2 to 0.3 years and a troposphere-to-stratosphere turnover time of 30 years. An EXAMS model of chloroform in water confirms other data suggesting that the major removal process for chloroform in water is evaporation.

The best analytical method for detection of chloroform appears to be gas chromatography with electron capture or electrolytic conductivity detection. This gives a detection limit of <5 ppt.

Pharmacokinetics

The pharmacokinetics and metabolism of chloroform have been studied in both humans and experimental animals. Chloroform is rapidly and extensively absorbed through the respiratory and gastrointestinal tracts. Absorption through the skin could be significant only in instances of contact with liquid chloroform.

The available data suggest that, in a human at rest, at least 2 hours are required to reach an apparent equilibrium of the body with the inhaled chloroform concentration. The magnitude of chloroform uptake into the body (dose or body burden) is directly proportional to the concentration of chloroform in the inspired air, the duration of exposure, and the respiratory minute volume.

The absorption of chloroform from the gastrointestinal tract appears to be virtually complete, judging from recovery of unchanged chloroform and metabolities in the exhaled air of humans and in the exhaled air, urine, feces, and carcass of experimental animals. Chloroform given in a corn oil vehicle to experimental animals is absorbed more slowly than chloroform given in water. Peak blood levels occurred at ≃1 hour after oral administration of chloroform in olive oil to humans or animals.

Following inhalation or ingestion exposure, the highest concentrations of chloroform are found in tissues with higher lipid contents. Results from the administration of ^{14}C-labeled chloroform to animals indicate that the distribution of radioactivity (reflecting both chloroform and its metabolities) may be affected by the route of exposure. Oral administration appeared to result in the accumulation of a greater proportion of radioactivity in the liver than did inhalation exposure. Differences in the distribution of chloroform and its metabolities between male and female animals were found only in mice and not in rats or squirrel monkeys. The kidneys of male mice accumulated strikingly more radioactivity than did those of female mice.

Chloroform is oxidized via microsomal cytochrome P-450 to trichloromethanol, which spontaneously dehydrochlorinates to the toxic and reactive intermediate compound, phosgene. The end products of the phosgene reaction with cellular water are CO_2 and hydrochloric acid, but significant amounts of phosgene and other reactive intermediates bind covalently to tissue macromolecules or conjugate with cysteine and glutathione. Covalent binding of the reactive intermediates to macromolecules is considered to be responsible for the hepato- and nephrotoxicity of chloroform. While the liver is the primary site for chloroform metabolism, other tissues, including the kidney, can also metabolize chloroform.

There is no evidence to suggest any qualitative difference for chloroform metabolic pathways in mice, rats, and humans. Interspecies comparisons of the magnitude of chloroform metabolism have been made only for the oral route. Metabolism of chloroform across species, including mice, rats, squirrel monkeys, and humans is proportional to the surface area of the species. The end metabolite, CO_2, is excreted in expired air. Dose-dependent pulmonary exhalation is the principal route of excretion for unmetabolized chloroform. Small amounts of chloroform metabolites are excreted in the urine and feces. Results from observations in humans suggest that chloroform metabolism is rate limited.

Regardless of the route of entry into the body, chloroform is excreted unchanged through the lungs and eliminated via metabolism, with the primary stable metabolite, CO_2, also being excreted through the lungs. High concentrations of unchanged chloroform have been found in the bile of squirrel monkeys after oral administration, but not in the urine or feces. The inorganic chloride generated from chloroform metabolism is excreted via the urine.

Decay curves for the pulmonary excretion of unchanged chloroform in humans appear to consist of three exponential components. The terminal component, thought to correspond to elimination from adipose tissue, had a half-time of 36 hours. This long half-time of chloroform residence in the human fat compartment indicates that fatty tissue concentrations of chloroform will not achieve steady-state equilibrium conditions with exposure concentrations until 6 to 7 days of continuous exposure to ambient concentrations, or longer for repetitive daily exposures in the workplace. Conversely, the long residence time of chloroform in the fat compartments of humans indicates that complete desorption of chloroform from these compartments requires 6 to 7 days in chloroform-free environs.

Health Effects Overview

Neurological, hepatic, renal, and cardiac effects have been associated with exposure to chloroform. These effects have been documented in humans as well as in experimental animals. In addition, studies with animals indicate that chloroform is carcinogenic and may be teratogenic.

Evidence of chloroform's effects on humans has been obtained primarily during the use of this chemical as an inhalation anesthetic. In addition to depression of the central nervous system, chloroform anesthesia was associated with cardiac arrhythmias (and some cases of cardiac arrest), hepatic necrosis and fatty degeneration, polyuria, albuminuria, and in cases of severe poisoning, renal tubular necrosis. When used for obstetrical anesthesia, chloroform was likely to produce respiratory depression in the infant. Humans exposed experimentally to chloroform for 20 to 30 minutes have reported dizziness, headache, and tiredness at concentrations >1000 ppm, and light intoxication at concentrations above 4000 ppm.

Similar symptoms occurred in workers employed in the manufacture of lozenges containing chloroform; exposure concentrations ranged from 20 to 237 ppm, with occasional brief exposure to ≃1000 ppm. Additional complaints were of gastrointestinal distress, and frequent scalding urination. The only other report of adverse effects stemming from occupational exposure to chloroform was of enlargement of the liver.

Acute inhalation experiments with animals revealed that single exposures to 100 ppm were sufficient to produce mild hepatic effects in mice. The exposure level that would produce mild renal effects is not known, but toxic effects occurred in the kidneys of male mice exposed to 5 mg/L (1025 ppm). In subchronic inhalation experiments, histological evidence of

mild hepato- and nephrotoxicity occurred in rats with exposures to as low as 25 ppm, 7 hours/day for 6 months. The effects were reversible if exposure was terminated, and did not occur when exposure was limited to 4 hours/day.

Information on the effects of acute and long-term oral exposure to chloroform is available primarily from experiments with animals. Human data are mainly in the form of case reports and involve the abuse of medications containing not only chloroform, but other potentially toxic ingredients as well; however, a fatal dose of as little as 1/3 ounce was reported. As with inhalation exposure, the primary effects of oral exposure were hepatic and renal damage. Narcosis also occurred with high doses. Subchronic and chronic toxicity experiments with rats, mice, and dogs did not clearly establish a no-effect level of exposure for systemic toxicity. Although a dose level of 17 mg/kg/day of chloroform produced no adverse effect in four strains of mice, the lowest dosage tested, 15 mg/kg/day, elevated some clinical chemistry indices of hepatic damage in dogs and appeared to affect a component of the reticuloendothelial system (histiocytes) in their livers.

No controlled studies have been performed to define dose-response thresholds for neurological or cardiac effects of ingested or inhaled chloroform. It is not known whether subtle impairment of neurological or cardiac function might occur at levels as low as or lower than those which affect the liver.

Several substances that are of interest because of accidental or intentional human exposure have been shown to modify the systemic toxicity of chloroform, usually by modifying the metabolism of chloroform to the reactive intermediate. Examples of substances that potentiate chloroform-induced toxicity are ethanol, PBBs, ketones, and steroids, while those that appear to protect against toxicity include disulfiram and high carbohydrate diets.

On the basis of presently available data, no definitive conclusion can be reached concerning the mutagenicity of chloroform. However, evidence from studies measuring binding to macromolecules, DNA damage, and mitotic arrest suggest that chloroform may be mutagenic.

Chloroform has the potential for causing adverse reproductive effects in pregnancy maintenance, delays in fetal development, and the production of terata in laboratory animals. The studies which administered chloroform by inhalation 7 hours/day reported more severe outcomes than other studies that administered chloroform by intubation, once or twice a day. The adverse effects produced in the conceptus were observed in association with maternal toxicity; however, the type and severity of effects appeared to be specific to the conceptus, affecting development to a much greater degree than the occurrence of maternal toxicity. It is concluded that chloroform is a potential developmental toxicant. The results of a preliminary study indicate that chloroform has no significant adverse behavioral effect on the fetus and produces embryotoxic effects only at maternally toxic levels.

The carcinogenic potential of chloroform has been experimentally evaluated in several animal species and by epidemiologic surveys including chronic animal studies. In all of these studies chloroform was administered by the oral route and not by inhalation. However, a carcinogenic response from chloroform exposure is not expected to be dependent upon the route of assimilation into the body although the magnitude of the response may vary.

Evidence for the carcinogenicity of chloroform in experimental animals includes: statistically significant increases in renal epithelial tumors in male Osborne-Mendel rats; hepatocellular carcinomas in male and female $B_6C_3F_1$ mice; kidney tumors in male ICI mice; and hepatomas in female Strain A mice and NLC mice. Chloroform has also been shown to promote growth and metastasis of murine tumors. In these cancer studies the carcinogenicity of chloroform is organ-specific, occurring primarily in liver and kidney, which are also the target organs of acute chloroform toxicity and covalent binding.

The carcinogenicity of chloroform in test animals was first investigated in 1945. Although the number of animals in each test group was small and the mortality was high at the higher doses, an increased incidence of hepatomas was observed in Strain A mice and confirmed in 1967 in a study using NLC mice. Chloroform was administered in oil by gavage in both studies.

In 1976, male and female $B_6C_3F_1$ mice, chloroform-treated by corn oil gavage, showed highly significant dose-dependent increases in hepatocellular carcinomas, with metastases to the lungs in some mice. In a similar study, statistically significant dose-dependent increases of kidney epithelial tumors were found in male Osborne-Mendel rats. In another study, kidney tumors were observed in male ICI mice administered chloroform in either toothpaste or arachis oil.

In the most recently published study (1985), chloroform administered in the drinking water of male Osborne-Mendel rats induced a statistically significant increase in the incidence of renal tumors, thus supporting the findings from the earlier study in which chloroform was adminstered in corn oil by gavage. Female $B_6C_3F_1$ mice, however, did not show an increase in the incidence of liver tumors when chloroform was administered in the drinking water. This was inconsistent with the positive findings reported in previous investigations of chloroform oil gavage treatment of mice. The lack of response of the mice in the drinking water study versus the highly significant response of these mice when chloroform was given in corn oil vehicle as a single bolus, suggests that chloroform-induced heptatocellular carcinomas in this strain of mice may be related to chloroform absorption patterns, the dosing regimen, peak blood levels of chloroform, and target tissue levels of its reactive intermediate metabolites. The corn oil carrier has not been shown to induce an increase in the incidence of liver tumors in mice.

Other studies of chloroform carcinogenicity have shown negative results. Treatment with a gavage dose of chloroform in toothpaste did not produce a carcinogenic response in female ICI mice or in male mice of the CBA, C57BL, and CF/1 strains, nor was a carcinogenic response observed in male or female Sprague-Dawley rats given chloroform in toothpaste by gavage, but early mortality was high in control and treatment groups. Gavage doses of chloroform in toothpaste did not cause a carcinogenic response in male and female beagle dogs treated for over 7 years, although there was an increased incidence of hepatic nodular hyperplasia. The daily chloroform doses given to mice and rats in toothpaste or arachis oil were lower than those given in corn oil or drinking water in studies showing a positive carcinogenic response. In newborn (C57 x DBA2-F1) mice given subcutaneous doses during the initial 8 days of life and observed for their lifetimes, a carcinogenic effect of chloroform was not evident. The doses levels used appeared well below a maximum tolerated dose and the period of treatment was quite short. In Strain A mice, chloroform was ineffective at maximally tolerated and lower doses in a pulmonary adenoma bioassay. However, other chemicals that have shown carcinogenic activity in different tests were ineffective in this particular Strain A mouse pulmonary

adenoma bioassay. Chloroform does not induce transformation of Syrian baby hamster kidney cells (BHK-21/C1 13) *in vitro*.

While no cancer epidemiologic studies have evaluated chloroform by itself, several studies have been made of populations with chlorinated drinking water, in which chloroform is the predominant chlorinated hydrocarbon compound. Small increases in rectal, bladder, and colon cancer were consistently observed by several case-control and ecological studies, several of which are statistically significant. Because other possible carcinogens were present along with chloroform, it is impossible to identify chloroform as the sole carcinogenic agent. Therefore, the epidemiologic evidence for the carcinogenicity of chloroform is considered inadequate.

It is generally accepted that the carcinogenic activity of chloroform resides in its highly reactive intermediate metabolities. Irreversible binding of chloroform metabolites to cellular macromolecules supports several theoretical concepts of the mechanism(s) for its carcinogenicity, including the possibility that chloroform may act as a promoter in animal tissues in addition to having complete carcinogen properties. Available data on chloroform metabolism and pharmacokinetics pertinent to the conditions of the carcinogenicity bioassays are used in the extrapolation of the dose-carcinogenic response relationships of laboratory animals to humans. There is no difference in absorption of chloroform across species. Also, there is no evidence to suggest any qualitative difference in the metabolic pathways or profiles of mice, rats, and humans for chloroform. An experimental basis exists for determining relative amounts of chloroform metabolized in various species, including man, and this information has been used in the unit risk derivation for chloroform.

Based on EPA's proposed Carcinogen Risk Assessment Guidelines, chloroform is classified as having sufficient animal evidence for carcinogenicity and inadequate epidemiologic evidence. The overall weight-of-evidence classification is group B2, meaning that chloroform is probably carcinogenic in humans.

The derivation of cancer risk values is based on the assumption of a nonthreshold mechanism for cancer induction, and consequently mathematical extrapolation models consistent with this assumption are utilized.

Five data sets are used to estimate the carcinogenic risk of chloroform. The end points include liver tumors in female mice, liver tumors in male mice, kidney tumors in male rats, and kidney tumors in male mice. The unit risk values at 1 mg/kg/day, calculated by the linearized multistage model on the basis of these data sets, are comparable. The risk value is useful for estimating the possible magnitude of the public health impact. The upper-bound incremental cancer risk derived from the geomatric mean of 4 data sets, chloroform gavage studies which showed a statistically significant increase of hepatocellular carcinomas in mice, is 8.1×10^{-2} per mg/kg/day. The carcinogen cancer assessment group (CAG) potency index for chloroform (defined as the slope x molecular weight) is 1×10^{1}, ranking it in the lowest quartile of 55 chemicals that the CAG has evaluated as suspect carcinogens. The upper-bound estimate of the incremental cancer risk due to ingesting 1 $\mu g/L$ of chloroform in drinking water is 2.3×10^{-6}. The upper-bound estimate of the incremental cancer risk due to inhaling 1 $\mu g/m^3$ of chloroform in air based upon positive gavage carcinogenicity studies is 2.3×10^{-5}. The upper-bound nature of these estimates is such that the true risk is not likely to exceed this value and may be lower.

Although the nonthreshold mathematical risk extrapolation model is conservative based upon a public health point of view, the correction used in the calculation of a human equivalent dose is scientifically conservative and may lead to an overestimate of the amount of chloroform metabolized in the test animals, and hence underestimate the risk. In addition, experimental data that include covalent binding in human tissues suggest that humans may have a greater than expected capacity to metabolize chloroform when compared to rodents, again indicating the possibility of underestimating the risk for humans.

This Project Summary was prepared by staff of Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

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The complete report, entitled "Health Assessment Document for Chloroform," (Order No. PB 86-105004; Cost: $36.95, subject to change) will be available only from:

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