Health Risk Assessment/Characterization of the Drinking Water Disinfection Byproduct Chloroform

This Document Was Prepared By:

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Under The Direction Of:

Health and Ecological Criteria Division
Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
Washington, DC 20460

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FOREWORD

The purpose of this document is to provide scientific support and rationale for the hazard identification and dose-response information pertaining to chronic oral exposure to chloroform. It is not intended to be a comprehensive treatise on the chemical or toxicology of chloroform. Matters considered in this risk characterization include knowledge gaps, uncertainties, quality of data and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

An earlier draft of this document underwent external peer review by three independent experts and experts within EPA. The charge to external peer reviewers and their comments are presented in the Appendix. Reviewers' comments were considered in preparing the version of this document released for public comment. Issues raised by the public during the comment period have been addressed in the final version of this document. Specifically, commenters stated that human studies were not considered in the chloroform assessment, and that risk to fetuses, infants, and children was not taken into consideration. To address these concerns, an expanded discussion of the human studies has been added. Concerns about risk to fetuses, infants, and children have been addressed by specifically discussing developmental, young animal, and reproductive toxicity of chloroform; by comparing the systemic toxicity of chloroform in these groups; and by considering differences between these groups and adults in the ability to activate chloroform to a toxic metabolite. A discussion of interactions among disinfectant byproducts and risk assessment for exposure to chloroform as part of a mixture has also been added in response to public comments. New papers noted by public commenters have also been included in this document.

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1. Introduction

Chloroform ($CHCl_3$) is a clear, colorless volatile liquid with a nonirritating odor and a sweet taste (Hardie, 1964 and Windholz, 1976). Some important physical and chemical properties of chloroform are summarized in U.S. EPA (1994). Chloroform was used as an anesthetic as early as 1847 but is no longer employed for this purpose. It is manufactured and used as a solvent and as an intermediate in the production of refrigerants, plastics and other solvents (U.S. EPA 1980).

Because of its volatility, chloroform has the potential for evaporation from water or other sources. Chloroform is stable in water, but light, aeration or the presence of metals such as iron promote degradation (Hardie, 1964). Chloroform is formed by the action of hypochlorous acid on endogenous organic molecules (e.g., humic or fulvic acids) present in the water.

A number of national drinking water surveys performed in the United States between 1975 and 1981 revealed that chloroform was detectable in a majority of systems using a surface water source. Average levels usually ranged from 20 to 90 ug/L (U.S. EPA 1975a, 1975b; Brass et al. 1977; U.S. EPA 1985). Chloroform was also detectable in systems using groundwater as a source, but usually at lower levels (1 to 10 ug/L). This is presumably because surface water typically contains higher levels of organic precursors than groundwater, and generally requires more extensive chlorination than groundwater. Concentration values have also been noted to vary as a function of season, being higher in warm weather and lower in cold weather (Wallace et al. 1987, 1988), probably due to reaction kinetics.

In 1994, U.S. EPA proposed the National Primary Drinking Water Regulations for Disinfectants and Disinfectant Byproducts (D/DBP). To support the regulation, EPA developed the following six Criteria Documents: Chlorine; Chlorine Dioxide, Chlorite, and Chlorate; Chloramine; Trihalomethanes; Chlorinated Acids, Aldehydes, Ketones, and Alcohols; and Bromate. The Agency is scheduled to publish the Final D/DBP Rule in November 1998. Since the 1994 proposal, several new studies on D/DBPs have become available. In order to provide an opportunity for public review and comment on these new data and how they might influence the proposed rule, EPA (October 10, 1997) published a text entitled "Summary of New Health Effects Data on Drinking Water Disinfectants and Disinfectant Byproducts (D/DBPs) for the Notice of Data Availability (NODA)" (U.S. EPA, 1997a).

In 1996, EPA proposed revisions to the 1986 U.S. EPA Guidelines for Carcinogen Assessment (U.S. EPA 1996). The proposed Guidelines include a new weight-of-evidence approach that emphasizes understanding the mode of action, conditions of expression of carcinogenicity (e.g., route and magnitude of exposure), and consideration of all other relevant data. The 1996 proposed Guidelines include several default procedures (linear, nonlinear, or both), rather than relying on the Linear Multi-Stage (LMS) model as the only default for extrapolation of dose-response relationships.

In 1996, EPA co-sponsored an International Life Sciences Institute (ILSI) project in which an expert panel was convened and charged with the following objectives:

- review the available database relevant to the carcinogenicity of chloroform and dichloroacetic acid (DCA), excluding exposure and epidemiology data;
- consider how end points related to the mode of carcinogenic action can be applied in the hazard and dose-response assessment;
- use guidance provided by the 1996 EPA Proposed Guidelines for Carcinogen Assessment to develop recommendations for appropriate approaches for risk assessment; and
- provide a critique of the risk assessment process and comment on issues encountered in applying the proposed EPA Guidelines (ILSI, 1997).

The panel was made up of 10 expert scientists from academia, industry, government, and the private sector. It should be emphasized that the ILSI (1997) report does not represent a risk assessment per se for chloroform (or DCA) but provides recommendations on how to proceed with a risk assessment for these two chemicals.

The ILSI (1997) expert panel considered a wide range of information on chloroform, including rodent tumor data, metabolism/toxicokinetic information, cytotoxicity, genotoxicity, and cell proliferation data. Based on its analysis of the data, the panel considered oxidative metabolism to be the predominant pathway of metabolism for chloroform, and that exposure to chloroform resulted in recurrent or sustained toxicity as a consequence of oxidative generation of highly tissue reactive and toxic metabolites [i.e., phosgene and hydrochloric acid (HCl)], which in turn would lead to regenerative cell proliferation. The panel considered this mode of action of chloroform as a key influence on the carcinogenic process, and concluded that chloroform was not acting through a direct DNA reactive mechanism. The ILSI report noted that the weight-of-evidence for the mode of action was stronger for the mouse kidney and liver responses, and more limited, but still supportive, for the rat kidney tumor responses. Thus, the ILSI (1997) panel viewed chloroform as a likely carcinogen to humans above a certain dose range, but considered it unlikely to be carcinogenic below a certain dose range, indicating that "This mechanism is expected to involve a dose-response relationship which is nonlinear and probably exhibits an exposure threshold." The panel recommended the margin of exposure approach (U.S. EPA, 1996) as appropriate for quantifying the cancer risk associated with exposure to chloroform.

The intent of this document is to integrate information from hazard identification, dose response assessment and exposure assessment, and synthesize an overall conclusion about the risk of chloroform that is informative and useful for decision makers. This document relies on secondary sources (e.g., U.S. EPA, 1994; U.S. EPA, 1997a) and certain key published literature, and considers the recommendations of the ILSI panel report (ILSI, 1997).

This report applies the principles of the 1996 proposed Guidelines to evaluate the new science that has emerged for chloroform. This report will strive to be clear, transparent, reasonable, and consistent with other risk characterizations of the U.S. EPA, using guidelines established by EPA (U.S. EPA, 1995) and others (Ohanian et al., 1997). EPA asked 3 external experts to review this report. These comments were considered in revising this text and are found in the Appendix. The revised document was released for public comment, and new data and issues raised by the public commenters are addressed in this final report.

2. Summary of Major Conclusions In Risk Characterization

2.1 Characterization of Hazard

2.1.1 Toxicokinetics

U.S. EPA (1994 and 1997a) and ILSI (1997) summarized data pertinent to the toxicokinetics of chloroform in part, as follows. Measurements of gastrointestinal absorption of trihalomethanes such as chloroform in mice, rats and monkeys indicate that absorption is rapid (peak blood levels at 1 hour) and extensive (64% to 98%). Limited data indicate that gastrointestinal absorption of chloroform is also rapid and extensive (at least 90%) in humans. Most studies of trihalomethane absorption have used oil-based vehicles and gavage dosing. One study in rats found higher chloroform blood levels following oral gavage administration of chloroform in water than after administration of chloroform in an oil vehicle. This was interpreted as being due to higher absorption from water than from oil, but the possible influence of differences in first-pass metabolism was not taken into account. Dermal absorption of chloroform in water by rats and hairless guinea pigs is rapid and extensive. Absorption has also been reported in humans dermally exposed to chloroform in water.

Absorbed chloroform appears to distribute widely throughout the body. For example, chloroform was detected in a number of postmortem tissues from humans, with highest levels (5 to 68 ug/kg) in body fat and lower levels (1 to 10 ug/kg) in kidney, liver, and brain. Radiolabeled chloroform was detected in a variety of tissues following oral dosing of rats and mice, with somewhat higher levels in stomach, liver, blood, and kidney than in lung, muscle, or brain. Chloroform was also rapidly distributed, after intraperitoneal injection, to the liver, kidney, and blood of male B6C3F_1 mice. Peak radioactivity levels in all three tissues were achieved within 10 minutes of dosing, and returned to background levels within 3 hours. Chloroform crosses the placenta and is detected in fetal tissues following inhalation exposure of pregnant rats.

Chloroform is extensively metabolized by both humans and animals. The main site of metabolism is the liver, but metabolism also occurs in the kidney. Recent studies have investigated the cytochrome P450 (CYP) isoenzymes responsible for trihalomethane (THM) metabolism. Chloroform is metabolized by both CYP2E1 and CYP2B1; however, due to different affinities of these enzymes for

chloroform, metabolism by CYP2E1 predominates at low doses. As doses increase, CYP2B1 begins to metabolize chloroform (Nakajima, 1995).

Both the oxidative and reductive metabolism of chloroform is mediated by these cytochrome P-450s. The oxidative pathway requires NADPH and oxygen, whereas the reductive pathway can utilize NADPH or NADH and is inhibited by oxygen. In the presence of oxygen (oxidative metabolism), the reaction product is trichloromethanol (CCL_3OH), which then decomposes to yield a reactive compound such as phosgene (CCl_2O). Phosgene is a reactive species, and may undergo a variety of reactions, including adduct formation with various cellular nucleophiles (histidine, tyrosine, methionine, and inositol), hydrolysis to carbon dioxide, or glutathione-dependent reduction to yield carbon monoxide. Reaction with nucleophiles in proteins or phospholipids can disable the affected molecules (enzymes, signal molecules, membrane phospholipids, etc.) which may lead to cytotoxicity or disruption of intracellular signaling. If oxygen is lacking (reductive metabolism), the metabolic reaction products appear to be free radical species such as dichloromethyl radical ($CHCl_2$ **!**). This radical is extremely reactive forming covalent adducts with a variety of cellular molecules and causing lipid peroxidation.

Although both oxidative and reductive pathways for chloroform metabolism have been described, the oxidative pathway is more important biologically. Reductive metabolism of chloroform to dichloromethyl radical appears to be a significant route of toxicity only at high doses and in induced animals. At high *in vitro* doses with male rat kidney microsomes, chloroform metabolism occurs primarily via the reductive pathway (Gemma et al., 1996a). Phospholipid adducts formed from the reductive metabolism of chloroform have also been observed *in vivo* (Gemma et al., 1996b), and the degree of reductive metabolism was a function of the oxygen tension, the chloroform dose, and the strain and species of the animal. However, when the relative importance of these pathways was evaluated by analysis of phospholipid adduct patterns (since the position of the adducted group on the phospholipid is an indicator of its source) a predominant role of oxidative metabolism in chloroform activation was seen at a low tissue dose, even at 5% pO_2, a concentration the authors considered to be representative of that found in the liver and kidney. At this oxygen level and a high tissue dose, phospholipid adducts indicative of both oxidative and reductive metabolism were found in extracts from the liver and kidneys of B6C3F1 mice. At the high tissue dose and 5% pO_2, extracts from the liver of Sprague-Dawley and Osborne Mendel rats produced adducts indicative of both oxidative and reductive metabolism, while kidney extracts produced primarily adducts related to reductive metabolism. It should be noted, however, that this was an *in vitro* assay using indirect indicators of oxidative and reductive metabolism. Under conditions of 20% oxygen partial pressure, less than 25% of phospholipid adducts in livers of B6C3F1 mice and the kidneys of DBA2 mice were to fatty acid tails, indicating that oxidative metabolism predominates at this oxygen level; other studies were conducted under anoxic conditions, but a hypoxic atmosphere was not investigated (Ade, 1994). Overall, the pattern of protein and lipid binding in the kidney microsomes correlated with hormonal status only under aerobic conditions. Therefore, oxidative metabolism of chloroform is implicated in the observed gender specificity of its kidney toxicity. These studies indicate that reductive metabolism may occur at low (but physiologic) oxygen tension in the liver and kidney, but only at high dose levels.

ILSI (1997) noted that the centrilobular region of the liver (the primary region for chloroform metabolism in the liver) is physiologically hypoxic. That document stated that oxygen partial pressures in the liver range from 1 to 60 mm Hg (0.1-8% pO_2), with a mean of approximately 20 mm Hg (2.6% pO_2) and the lowest values in the centrilobular region. Gemma et al. (1996a) also considered a pO_2 of 5% to be representative of physiological kidney oxygen levels. However, ILSI (1997) noted that "a large amount of circumstantial evidence argues against the significance of the anaerobic pathway to chloroform under normal conditions." This circumstantial evidence includes the following: (1) Macromolecular binding (resulting from reductive metabolism) following chloroform administration accounts for only a very small portion of the delivered dose. (2) Compared to other haloalkanes, chloroform is relatively ineffective as a source of free radicals. (3) The closely related compound carbon tetrachloride (CCl_4) is a potent hepatotoxicant and is metabolized almost exclusively by the reductive pathway, but the available data indicate that carbon tetrachloride carcinogenicity does not result from direct mutagenicity. (4) Chloroform and carbon tetrachloride differ substantially in the mechanisms of action related to the necrotic lesion and the kinetics of damage. Based on these considerations, ILSI (1997) concluded that "free radicals do not play a significant role in chloroform toxicity or carcinogenicity."

Both *in vivo* and *in vitro* studies indicate that the pattern of trihalomethane metabolism may differ between animal species and sexes. *In vivo,* mice have been found to metabolize trihalomethanes to carbon dioxide more extensively than do rats (40% to 80% versus 4% to 18%). *In vitro*, the capacity for reductive metabolism of trihalomethanes has been found to be greater in hepatic microsomes from mice than rats. In addition, both total metabolism and the formation of covalent adducts in renal microsomes have been found to be greater in male mice than female mice. These metabolic differences may explain some of the important toxicological differences that have been noted between sexes and species.

Excretion of chloroform occurs primarily via the lungs, with greater amounts excreted unchanged as dose increases and metabolism is saturated. In humans, approximately 90% of an oral dose of radiolabeled chloroform was exhaled as the end metabolite, carbon dioxide, or as the parent compound, chloroform. Levels in the urine were below the limit of detection (0.1%). In mice and rats, 45% to 88% of an oral dose of chloroform was excreted from the lungs either as chloroform or as carbon dioxide, with 1% to 5% excreted in the urine. Intraperitoneal injection of rats with ^{36}Cl-chloroform resulted in the appearance of both inorganic and organic forms of chloride in the urine, but the total amount was not quantified.

No data were located regarding the bioaccumulation and retention of chloroform following chronic exposure. However, based on the rapid metabolism and excretion of chloroform, along with the low levels of chloroform in human autopsy samples, marked accumulation and retention is not anticipated.

2.1.2 Health Effects of Exposures in Humans

EPA (1994 and 1997a) also summarized the health effects of exposures in humans as follows. In a case study of a young man who ingested 4 ounces of chloroform (a dose of about 2,500 mg/kg), prominent clinical findings included jaundice, an enlarged liver, increased serum levels of bilirubin, alkaline phosphatase (AP), and serum glutamic oxaloacetic transferase (SGOT), along with albuminuria, glucosuria, ketonuria and the presence of red cells and granular casts in the urine. These observations indicated that in humans, as in other animals, the liver and kidneys are the organs most affected by acute chloroform ingestion.

Workers exposed to chloroform by inhalation at levels of 112 to 1,158 mg/m^3 for 1 or more years complained of nausea, lassitude, dry mouth, flatulence, thirst, depression, irritability, and "scalding" urination, but clinical examination and tests of liver function (serum enzyme levels) failed to detect any abnormalities. Inhalation exposure of workers to chloroform at levels of about 10 to 1,000 mg/m^3 for 1 to 4 years was reported to be associated with an increased incidence of viral hepatitis and enlarged liver.

Although a number of studies have investigated the potential association between chlorinated drinking water and cancer or developmental effects, only a few of these evaluated the degree of association with chloroform exposure. Some epidemiological studies suggest there may be an association between ingesting chlorinated water and increased cancer mortality rates. However, because chlorinated drinking water contains a number of disinfectant byproducts, and, depending on the source, may contain other contaminants, it is usually not possible to associate any increased cancer rate with a specific contaminant. Other limitations common to most or all of the studies include (1) the potential for chloroform to serve as a surrogate measure of other contaminants that might be truly responsible for the observation in the study, (2) insufficient historical exposure information, (3) potential misclassification of exposure due to use of county-wide or region-wide exposure information, and (4) insufficient accounting for migration.

In the most thorough evaluation of the potential link between chloroform in drinking water and cancer, Doyle et al. (1997) reported an association between chloroform ingestion in drinking water and the development of colon cancer in a study of 28,237 postmenopausal Iowa women; there was no association with the other cancer types evaluated. (Note that the study population was very specific and was not representative of the population of colon cancer cases in the U.S.) In this cohort study, women aged 55-69 completed a mail survey in 1986 on medical history, anthropomorphic data, and risk factors. The cohort members were followed for cancer incidence through the state health registry, and for cancer mortality through the National Death Index and questionnaires mailed in 1987, 1989, and 1992. Based on the follow-up surveys, out-migration was estimated at less than 1% annually. Drinking water source was determined in the 1989 mail survey. Only women drinking municipal water or private well water for more than 10 years were included in this study. Exposure to four trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) was

based on state-wide surveys in 1979 and 1986 of municipal water supplies, and exposure levels were linked to cohort members on a community basis. Cohort members for which there was no trihalomethane exposure information, who reported a change in residence, or who reported a prior diagnosis of cancer (other than skin cancer) were excluded from the study. All relative risks were adjusted for such potential confounders as smoking status, fruit and vegetable intake, and age.

The authors focused on the relationship between chloroform exposure and cancer, since chloroform was the most commonly occurring trihalomethane, had the broadest concentration range, and correlates well with the concentration of the other trihalomethanes. Private well users were excluded from the analysis, since trihalomethane levels were not available, bringing the size of the cohort analyzed to 19,199. For the 1986 exposure data, there was a dose-related increase in colon cancer with increasing chloroform levels, with relative risks of 1.06, 1.39, and 1.68 at 1-2 ug/L, 3-13 ug/L, and 14-287 ug/L, respectively (trend test $p<0.01$). The increase was statistically significant in the high-dose category (95% confidence interval 1.11-2.53). The association remained when the analysis was limited to women who had used the current water source for more than 20 years, and was strengthened when 1238 women reporting a history of colorectal polyps at baseline were excluded. A significant ($p<0.01$) trend for total cancer with increasing chloroform levels was also observed, with statistically significant effects in the mid- and high-dose groups; this increase was attributed to the increase in colon cancer. Similar results were obtained with the 1979 exposure data, although that data included fewer communities. No statistically significant associations between colon cancer and the brominated trihalomethanes were found. However, the study authors noted that the levels of those compounds was lower than that of chloroform, with a large number of sources with undetectable levels, leading to unstable relative risk estimates. Since chloroform is one of many water contaminants and one of many disinfection byproducts, it may have served as a surrogate for the causally responsible contaminant(s). The study authors also noted that some exposure misclassification may have occurred, since exposure information prior to 1986 was not available. In addition, no information on water consumption was available, and the reporting of chloroform levels was only on a community basis. Finally, the study was conducted only on women, although the authors stated that the fundamental biology of colon carcinogenesis would be expected to be similar for men and women.

In a case-control study of colorectal cancer in New York state, no relationship between cumulative chloroform exposure and cancer was found in a logistic analysis that controlled for source type, population density, marital status, age, and year of death (Lawrence et al., 1984). In a study of 400 residents of Telemark, Norway, age 50-59 years, the prevalence of colorectal polyps was not associated with the concentration of chloroform in the drinking water (Hoff et al., 1992).

A few studies have also investigated the potential association between chloroform in drinking water and developmental effects. As discussed in U.S. EPA (1994), Kramer et al. (1992) conducted a population-based case-control analysis in Iowa to determine if exposure to trihalomethanes was associated with low birthweight, prematurity, or intrauterine growth retardation. After adjusting for maternal age, number of previous children, marital status, education, adequacy of prenatal care, and

maternal smoking, the authors found a statistically significant association between exposure to water chloroform levels of at least 10 ug/L and intrauterine growth retardation. The association remained when the only water source was deep wells, a source unlikely to be contaminated with pesticides and other chemicals, aside from disinfection byproducts. The study authors noted that chloroform may have been a marker for other organic halides. A recent epidemiological study found an association between spontaneous abortions and drinking water levels of total trihalomethanes (THMs) or of the THM bromodichloromethane (BDCM) (Waller et al., 1997). No association with chloroform levels was found.

No human data were located regarding whether chloroform exposure may result in greater risk for any human subpopulation than for the general population. However, reasonable predictions of sensitive populations can be made based on animal data. Based on the induction of CYP2E1 by ethanol, alcohol consumption may increase toxicity in the liver. Based on potentiation of chloroform-induced hepatotoxicity in a rat model of diabetes, diabetics may be also more sensitive to the liver effects. People with pre-existing kidney or liver damage may also be more sensitive, since they would be expected to have a lower functional reserve capacity. On the other hand, decreased liver function might result in decreased capacity for chloroform metabolism, which would result in decreased sensitivity.

Based on a detailed consideration of the animal data on the systemic and developmental toxicity of chloroform, and of the metabolism of chloroform at different ages, fetuses, infants, and children are not considered sensitive subpopulations for chloroform toxicity. The supporting data are addressed in Section 2.1.3 in the context of the animal data.

2.1.3 Health Effects of Exposure in Experimental Systems

Data from animal studies also are summarized in EPA (1994 and 1997a) as follows. Large oral doses of chloroform are lethal to laboratory animals, with acute LD_{50} values ranging from 119 to 2,000 mg/kg. Death from acute high-dose chloroform exposure was usually found to be due to central nervous system depression and cardiac effects, and was usually accompanied by histopathological changes in the liver and kidney.

Acute oral exposure to sublethal doses of chloroform can also produce effects on the liver, kidney, and central nervous system. In mice, single oral doses of 60 to 89 mg/kg produced kidney damage, with doses of 140 to 250 mg/kg producing liver damage. Organ damage was characterized by fatty infiltration, cellular necrosis, vacuolization, enzyme level changes, and/or organ weight changes. Ataxia and sedation were noted in mice receiving 500 mg/kg chloroform.

Short-term exposures of laboratory animals to chloroform have been observed to cause effects on the liver, kidney, central nervous system, and immune system. Hepatic effects, including organ weight changes, elevated serum enzyme levels, and histopathological changes were reported in mice

and/or rats administered 37 to 290 mg/kg-day chloroform. Kidney effects, characterized by decreased para aminohippurate (PAH) uptake, histopathological changes and organ weight changes, were reported in mice and/or rats administered 37 to 148 mg/kg-day chloroform.

The predominant effects of longer-term oral exposure to chloroform occur in the liver and kidney. The effects on these two organs are similar to those described for short-term exposures. Hepatic effects were reported in mice, rats and dogs administered 15 to 180 mg/kg-day chloroform. In general, these dose ranges are slightly lower than those reported to cause effects following short-term exposures.

Data concerning the developmental effects of chloroform indicate toxicity to the mother and fetus at high doses and suggest that reproductive and developmental toxicity may occur as well. Signs of maternal toxicity (decreased body weight and changes in organ weight) were reported in rats, rabbits and/or mice administered 50 to 100 mg/kg-day chloroform. Fetotoxicity, as indicated by decreased fetal body weights, was evident in the offspring of rats administered 121 to 400 mg/kg-day chloroform. Delayed ossification and sternebral aberrations have been reported in rats and/or rabbits administered 20 to 200 mg/kg-day chloroform. Statistically significant malformations and variations (cleft plate, imperforate anus, acaudia, delayed ossification) have been observed in inhalation studies in which mice and/or rats were exposed to 30 or 100 ppm chloroform (either 147 or 488 mg/m^3, respectively).

The overall evidence regarding chloroform genotoxicity is mostly negative. *In vitro*, chloroform has yielded mixed but mainly negative results in a number of assays of mutagenic activity, including 30 of 34 point mutation assays. Some of these results, however, are inconclusive because of inadequacies in experimental protocols, especially in the failure to use an appropriate (reconstituted) activation system or to take precautions to prevent the escape of volatilized chloroform (U.S. EPA 1985; Rosenthal, 1987). *In vitro* assays for early evidence of DNA damage (sister chromatid exchanges or DNA damage in yeast) tend to give positive results, but these endpoints may not be indicative of DNA alkylation and mutation. For example, the sister chromatid exchange assay has a low specificity for predicting carcinogenesis (i.e, a high rate of false positives compared to results of the rodent cancer bioassay). Thus, the weight of the evidence indicates that chloroform is not a DNA-reactive mutagen.

Recent studies also found that chloroform was negative in bacterial mutagenesis assays (Roldan-Arjona and Pueyo, 1993; LeCurieux et al., 1995), although a positive response was seen at very high doses with a strain engineered to produce endogenous glutathione S-transferase (Pegram et al., 1997). Chloroform was negative in *in vivo* and *in vitro* UDS assays (Larson et al., 1994d). Chloroform was reported as positive in the presence of S9 in an SCE assay in a rat leukemia cell line (Fujie et al., 1993). Positive results were obtained in a mouse micronucleus test (Shelby and Witt, 1995). Butterworth et al. (1998) found no statistically significant increases in mutant frequency in *lacI* transgenic B6C3F1 mice exposed to chloroform via inhalation, although the chloroform-exposed groups exhibited a consistent, dose-related increase over controls. This system has the advantage of ease in identification of *in vivo* mutations. Exposure was to a nonhepatotoxic (10 ppm), mildly

hepatotoxic (30 ppm), or overtly hepatotoxic (90 ppm) concentration, and mutant frequencies were evaluated after 10, 30, 90, or 180 days of exposure.

Chloroform also causes the development of both benign and malignant tumors in animals. The following summary derives in part from ILSI (1997). Several bioassays have been performed that sought to characterize the potential for liver carcinogenicity in mice. When given by corn oil gavage, chloroform provided a positive carcinogenic response in the male and female mouse liver (NCI, 1976).[1] Time-weighted average doses in this study were 0, 138, and 277 mg/kg-day for males and 0, 238, and 477 mg/kg-day for females. Corresponding incidences of liver tumors were 1/18 (6%), 18/50 (36%), and 44/45 (98%) for males, and 0/20 (0%), 36/45 (80%), and 39/41(95%) in females. Mice dosed by drinking water (Jorgenson et al., 1985)[2] or by inhalation (Matsushima, 1994),[3] however, failed to exhibit a liver tumor response even though the doses were similar to those of the

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[1] As described in EPA (1998), in a gavage bioassay (NCI, 1976), Osborne-Mendel rats and B6C3F1 mice were treated with chloroform in corn oil 5 times/week for 78 weeks. Fifty male rats received 90 or 125 mg/kg-day; females initially were treated with 125 or 250 mg/kg-day for 22 weeks and 90 or 180 mg/kg-day thereafter. Male mice received 100 or 200, raised to 150 or 300 mg/kg-day at 18 weeks; females were dosed with 200 or 400, raised to 250 or 500 mg/kg-day. A significant increase in kidney epithelial tumors was observed in male rats and highly significant increases in hepatocellular carcinomas in mice of both sexes. Liver nodular hyperplasia was observed in low-dose male mice not developing hepatocellular carcinoma.

[2] As described in EPA (1998), Jorgenson et al. (1985) administered chloroform (pesticide quality and distilled) in drinking water to male Osborne-Mendel rats and female B6C3F1 mice at concentrations of 0, 200, 400, 900, and 1800 mg/L for 104 weeks. These concentrations were reported by the author to correspond to doses of 0, 19, 38, 81, and 160 mg/kg-day for rats and 0, 34, 65, 130, and 263 mg/kg-day for mice. A significant increase in renal tumors in rats was observed in the highest dose group. The increase was dose related. The liver tumor incidence in female mice was not significantly increased. This study was specifically designed to measure the effects of low doses of chloroform in drinking water.

[3] This recent, and as yet unpublished, work of Matsushima (1994) states that groups of 50 male and 50 female F344 rats were exposed to chloroform vapor, 6 hours per day, 5 days per week, for 104 weeks. Exposure concentrations were 0, 10, 30 or 90 ppm. Groups of 50 male and 50 female BDF1 mice were exposed on the same schedule. The final exposure concentrations for the mice were 0, 5, 30, or 90 ppm. Preliminary studies showed that BDF1 mice (especially males) were initially sensitive to the toxic effects of chloroform, but that exposed animals later developed resistance to the chemical. Therefore, animals in the 30 ppm groups (both males and females) were exposed in a series of increasing concentrations, as follows: 5 ppm for 2 weeks, 10 ppm for 2 weeks, and 30 ppm for 100 weeks. Similarly, animals in the 90 ppm groups were exposed to 5 ppm for 2 weeks, 10 ppm for 2 weeks, 30 ppm for 2 weeks, and 90 ppm for 98 weeks. A preliminary report suggests treatment related kidney neoplasia only in male mice, with a small but statistically significant increase in liver neoplasia in females if the incidences of carcinomas and adenomas were combined.

corn oil gavage study. From these studies, it appears that neither the daily dose, nor the cumulative dose of chloroform, was predictive of tumor outcome in the drinking water study in mice. The inhalation study was negative in the liver despite a concentration escalation strategy used to achieve final concentrations that exceeded acutely lethal concentrations by several fold.

As also discussed in ILSI (1997), several bioassays have been performed that sought to characterize the potential for liver carcinogenicity in rats. The only study in which an increased incidence of liver tumors was observed was conducted with chloroform administered in drinking water, and the tumors were found only in female Wistar rats, not in males (Tumasonis et al., 1987). The interpretation of these results is complicated by the small size of the control group and the longer survival of the treated (185 weeks) versus control females (145 weeks). Other drinking water, corn oil gavage, and inhalation studies in various rat strains failed to demonstrate an increased incidence of liver neoplasia.

As also discussed by ILSI (1997), chloroform exposure produced positive kidney tumor responses in both male rats and mice, but not in females of either species. For example, a significant increase in renal tumors in male BDF1 mice was seen following inhalation exposures (Matsushima, 1994) and in male ICI mice exposed to chloroform either in an arachis oil base or in toothpaste (Roe et al., 1979). Although renal tumors were not found in male B6C3F1 mice following chloroform exposure by corn oil gavage (NCI, 1976) or drinking water (Jorgenson et al., 1985), positive responses for renal tumors were found in male Osborne-Mendel rats. The incidences of all kidney tumors in this strain of rats was 1/50 (2%), 6/313 (2%), 7/148 (5%), 3/48 (6%), and 7/50 (14%) for exposures of 0, 200, 400, 900, and 1800 ppm of water, respectively. F344 and Sprague-Dawley rats did not, however, show this tumor response. Thus, responses in kidney tumors appear to vary with route of exposure, administration vehicle, and strain of rodent. This makes it difficult to develop conclusive statements regarding potential strain differences in tumor response. Nonetheless, it is clear that when a response is observed, males are more responsive than females. This sex-specific difference is consistent with the observation that kidney metabolism of chloroform is higher in males than in females.

Male F344 rats were exposed to chloroform in drinking water for 100 weeks at concentrations of 0, 900, or 1,800 ppm as described in a recent, and as yet unpublished, work (DeAngelo et al., 1995). Interim sacrifices of groups of 6 animals were performed at 26, 52 and 78 weeks, and groups of 50 animals were scheduled for the 100 week sacrifice. At each time point, the liver and kidney were examined for gross and microscopic lesions. In the liver, with the exception of midzonal vacuolization (probably due to fat accumulation), no lesions other than those normally associated with aging rats were reported at any of the sacrifice periods. A preliminary report suggested an increase in liver tumors at the 1800 ppm dose. Kidney tumors were not found at either dose.

2.1.4 Children's Risk Issues

Three questions were considered in evaluating whether fetuses and children are more sensitive than adults to chloroform exposure:

- Are fetuses and children more susceptible than adults to the systemic toxicity of chloroform?
- Does chloroform causes reproductive and developmental effects at doses below those causing systemic toxicity (including cancer)?
- How does the ability of fetuses and children to metabolize chloroform to a toxic metabolite compare with that of adults?

Addressing the first question, whether fetuses and children are more sensitive than adults to the hepatotoxic effects of chloroform, is complicated by the absence of studies in which systemic effects in young rats were completely evaluated. An ideal study to examine this question would include exposure *in utero*, during lactation, and between weaning and reproductive age, with histopathological evaluation shortly after weaning and before reproductive age. Such a study design would eliminate the potential for repair during adulthood of any damage sustained by the juvenile, and would detect systemic effects of chloroform on the developing organism. (Teratogenic effects of chloroform are detected by developmental studies, as discussed with regard to the second question.) However, in the absence of such a study for chloroform, some comparisons can be made based on the available studies. In particular, direct comparisons can be made between two mouse studies in which chloroform was administered by corn oil gavage. In the first study (NTP, 1988), CD-1 (ICR)BR mice were exposed to chloroform *in utero*, during lactation, and then by gavage as young rats through "young" adulthood. Mild to moderate liver histopathology (degeneration of centrolobular hepatocytes, accompanied by occasional single cell necrosis) was observed in females at 41 mg/kg-day, the only dose at which systemic effects were evaluated. No adverse effects on fertility or reproduction of the F_1 generation were observed. Although there was no significant effect on sperm quality (sperm motility, density, and percent abnormal sperm) of the F_1 males, vacuolar degeneration of ductal epithelium in the cauda epididymidis was observed at 41 mg/kg-day. Thus, the only dose tested in this study, 41 mg/kg-day, was a LOAEL for liver histopathology.

In the second study (Bull et al., 1986), 6-8 week old B6C3F1 mice were exposed to 60, 130, or 270 mg/kg-day for 90 days by gavage in either corn oil or Emulphor (an emulsifier). This discussion focuses on the corn oil gavage results, to facilitate direct comparison to the NTP (1988) study. Liver histopathology (extensive vacuolation of the liver accompanied by lipid accumulation) was observed at a dose of 60 mg/kg-day after chloroform was administered by corn oil gavage, but higher corn oil gavage doses had corresponding less accumulation of lipid. A 10% decrease in body weight and a 20% (females) to 30% (males) increase in relative liver weight were also observed at the low dose, with larger effects at the higher doses. Based on these effects, the study LOAEL could be considered to be the low dose, 60 mg/kg-day. (This LOAEL differs from that in the Trihalomethanes Criteria Document [U.S. EPA, 1994]. That document considered 130 mg/kg-day to be a NOAEL, apparently because of the differences in toxicity evoked by chloroform when administered by corn oil or Emulphor gavage, and the related difficulty in making judgments regarding the critical effect.)

The similarity of effects at comparable corn oil gavage doses in the 2-generation study (exposure of fetuses to young adults) and the 90-day study (exposure of young adults to adults) indicates that there is no substantial additional sensitivity attributable to exposure of the former group. However, two factors limit the strength of this conclusion: (1) Different strains were evaluated; and (2) Neither study identified a NOAEL. On the other hand, exposure of the F_1 generation in the NTP study was somewhat longer than the 90-day exposure in the subchronic study, strengthening the conclusion that fetuses and children are not more sensitive.

The second question, whether reproductive and developmental effects occur at doses below those causing systemic toxicity, can be evaluated by directly comparing effect levels for reproductive/developmental effects and for systemic toxicity. No effects of chloroform on reproductive function have been identified (NTP, 1988). Oral developmental toxicity studies have found decreased fetal weight (Thompson et al., 1974) and inhalation developmental studies have found an increased incidence of delayed ossification (Baeder and Hofmann, 1991), but these effects occurred at doses above those causing hepatotoxicity. Thus these effects do not constitute the critical effect, and so the RfD based on liver effects would be sufficiently protective. Based on an increased incidence of fetuses with incompletely ossified skull bones in rabbits (Thompson et al., 1974), the NOAEL for developmental toxicity is 35-50 mg/kg-day. It should be noted, however, that a definitive NOAEL could not be identified in that study, due to the absence of a clear dose-response. The developmental NOAEL is at least three times higher than the LOAEL of 12.9 mg/kg-day for hepatotoxicity in dogs that forms the basis for the RfD. Therefore, the RfD based on hepatotoxicity in dogs is also adequate for protection from developmental effects.

The third question concerns the relative ability of fetuses, children, and adults to metabolize chloroform to a toxic metabolite. Because the toxicity of chloroform is dependent on oxidative metabolism, primarily by cytochrome P450 CYP2E1, studies on CYP2E1 levels in fetal and adult tissues were evaluated to determine whether fetuses would be expected to be more sensitive than adults to the effects of chloroform. The status of CYP2E1 in fetuses remains unclear, with conflicting studies. Most of the existing studies indicate that this enzyme is expressed in human adults but not in human fetuses, even when measured using sensitive assays (reviewed in Hakkola et al., 1998). In these studies, levels of both CYP2E1 protein and of the associated enzyme activity were undetectable before birth, but rose rapidly shortly after birth, due to stabilization of the CYP2E1 protein. However, at least three studies indicate CYP2E1 is expressed in fetal liver or cephalic tissue (Boutelet-Bochan et al., 1997; Carpenter et al., 1996; Vieira et al., 1996). Boutelet-Bochan et al. (1997) detected low levels of *Cyp2e1* mRNA transcription in human fetal brains (gestation days 52-117, or 7-17 weeks), and levels tended to increase with gestational age. However, transcription was detected only using a very sensitive assay (reverse transcriptase-polymerase chain reaction - RT-PCR) or the moderately sensitive RNase protection assay. Transcription in fetal liver was much lower, and was detectable in only two of six samples. Also using the RNase moderately sensitive technique, Carpenter et al. (1996) found transcription of *Cyp2e1* mRNA in the liver of human fetuses at 19-24 weeks gestation, but not at 10 weeks gestation. Fetal liver microsomes could metabolize the CYP2E1 substrate ethanol, but at a rate

only 12-27% of adult liver microsomes. At least most of the observed activity was specific to CYP2E1, since it was inhibited by an anti-CYP2E1 antibody. Like adult hepatocytes, fetal hepatocytes exposed to ethanol had induced levels of CYP2E1. Vieira et al. (1996) found that CYP2E1 protein could not be detected immunochemically in fetal human liver, and there was only minimal evidence of *Cyp2E1* mRNA or CYP2E1 activity in fetal liver microsomes. (The difference in assay results may be due to differences in sensitivity, or to cross-reaction of CYP1A1 activity.) The authors found, however, that CYP2E1 protein levels rise rapidly in the first few hours after birth, with a slow increase in protein levels and in CYP2E1 RNA levels during childhood.

Thus, the overall human data show that if CYP2E1 activity exists in human fetuses, levels are much lower than those in adults. Regardless of fetal CYP2E1 expression, the enzyme is rapidly induced upon birth. For this reason, children would be expected to be capable of chloroform metabolism, although the amount of CYP2E1 may be less than that present in the adult. Overall, the data on CYP2E1 activity provide no evidence to suggest that children are more susceptible than adults.

The animal studies of developmental CYP2E1 regulation provide uniform evidence of the rapid induction of this gene soon after birth (Song et al., 1986; Umeno et al., 1988; Schenkman et al., 1989; Ueno and Gonzalez, 1990). The idea that the enzyme activity peaks before weaning with a gradual decrease to adult levels suggested by some scientists, however, has not been consistently reported in the three studies which compared expression over this period of time.

For example, Schenkman et al. (1989) indicate that CYP2E1 protein is present in low levels in neonates, rises to a peak level at age 2 weeks, and subsequently decreases to adult levels by puberty. Analysis of protein levels quantified from western blots showed a maximum at 2 weeks with decreasing levels at 4 and 12 weeks. The protein level at 12 weeks was approximately 50% of the level at 2 weeks. The authors did not provide a statistical analysis of this result, but it appears from the error bars that the 2-week and 12-week levels (but not 4 weeks levels) were significantly different.

Song et al. (1986) conducted a similar analysis and reported a rapid transcriptional induction of *Cyp2e1* (P450j) within 1 week following birth which remained elevated throughout 12 weeks. The authors did not quantitate the western blots, but visual inspection indicates a small decline in protein levels by 12 weeks. However, in this same study, enzyme activity gradually increased over time, reaching a maximum at adulthood. Nor was an age-dependent decrease in mRNA levels observed.

Ueno and Gonzalez (1990) showed that extracts from 3 day old and 12 week old rat liver, but not fetal or newborn rat liver were able to generate significant *Cyp2e1* transcription in vitro. The ability of the extract to drive transcription of *Cyp2e1* was slightly greater at 12 weeks.

If the two-fold increase in *Cyp2e1* induction in animals where verified, its importance in terms of chloroform toxicity would depend on the dose. Under low dose conditions (for example, much lower than the Km) it is possible that an increase in the level of enzyme would not have any effect on active

metabolite formation since the amount of chloroform, and not CYP2E1, would control the rate of the enzyme activity. On the other hand, under saturating doses of chloroform, all the available enzyme would be active, thus a two-fold increase in CYP2E1 could result in greater activation of the compound. Additional analysis of the expected dose relative to the levels of enzyme could help elucidate the potential for these differing scenarios to occur.

Taken together, these animal studies do not provide conclusive evidence of an early period of increased enzymatic activity in young animals when compared with adults. While the animal data remain unclear regarding the potential for a period of increased CYP2E1 activity above that in the adult, for humans, a gradual increase of CYP2E1 activity throughout out childhood with a maximum level at adulthood, as described by Hakkola et al. (1998).

2.1.5 General Mechanism of Toxicity

U.S. EPA (1994 and 1997a) and ILSI (1997) summarized information on the potential mechanism of toxicity for chloroform, as follows.

U.S. EPA (1994 and 1997a) summarized three lines of evidence that indicate that chloroform metabolism is essential for toxicity: (1) the tissues that most actively metabolize chloroform (liver, kidney) are also the chief target tissues; (2) chemical treatments that increase or decrease metabolism also tend to increase or decrease toxicity in parallel; and (3) species- and sex-related differences in metabolism are paralleled by similar differences in toxicity. The detailed biochemical mechanism by which chloroform metabolism leads to toxicity are not certain, but covalent binding of reactive metabolites to cellular macromolecules is very likely a component. Such metabolites are produced by oxidative metabolism to dihalocarbonyls and perhaps by reductive metabolism to free radicals. Free radical production may also lead to cell injury by inducing lipid peroxidation in cellular membranes.

ILSI (1997) also used information on strain-specific differences in chloroform metabolism to explain apparently contradictory bioassay data. As noted in Section 2.1.3, renal tumors were observed in male BDF1 mice exposed to chloroform via inhalation (Matsushima, 1994) and in male ICI mice exposed to chloroform in a toothpaste or arachis oil vehicle (Roe et al., 1979), but not in male B6C3F1 mice administered chloroform by corn oil gavage (NCI, 1976) or in drinking water (Jorgenson et al., 1985). ILSI (1997) noted that males of the DBA strain, from which BDF1 mice were derived, have higher tubular levels of the enzymes that bioactivate chloroform, and are much more susceptible to chloroform-induced renal damage, than male C57BL mice, the parental strain for B6C3F1 mice. Thus, the observed data for kidney tumors in mice are consistent with the hypothesis that the chloroform is metabolized to a cytotoxic compound, and that the resulting toxicity and cell proliferation can result in the development of chloroform-induced cancer.

Formation of DNA adducts has not been shown with chloroform exposure. Although the formation of DNA adducts is the traditional hypothesis of tumor formation, the weight of evidence

favors the hypothesis that carcinogenesis may be related to increased cell proliferation following direct tissue injury. This is the hypothesis suggested by ILSI (1997) and Golden et al. (1997). However, this latter hypothesis has not been definitively linked to chloroform carcinogenesis.

Several chemicals, including various ketones, dichloroacetic acid, and carbon tetrachloride, potentiate the toxic effects of chloroform. The mechanism(s) of the potentiation by ketones is not known, but appears to include a process other than induction of microsomal enzymes. The vehicle (corn oil versus aqueous) used for oral dosing also affects toxicity, with toxicity generally being more severe following administration in corn oil. The difference in toxicity may also be due to a difference in dose rate of a bolus dose following the gavage (in oil) versus the more extended nature of exposure in drinking water (several hours).

Larson and coworkers have conducted a series of studies (Larson et al. 1993, 1994a, 1994b, 1994c, 1995a, 1995b, 1996; Templin et al. 1996a, 1996b, 1998) investigating the relationship among chloroform exposure, cytotoxicity, regenerative cell proliferation, and histopathology in rats and mice. Effects of oral exposure were investigated in a series of short-term studies of 1 to 21 days; inhalation exposures were conducted for 4 to 90 days. Based on earlier experiments, the study authors assumed that all increases in cell division, as measured by the labeling index, were due to regenerative cell proliferation, and thus an indirect measure of cytotoxicity. The labeling index is defined as the percentage of cells in a tissue that are in S-phase (that portion of the cell cycle when DNA synthesis occurs in preparation for cell division) within a specified time interval. The value of the labeling index is usually low (<4%) in tissues such as liver and kidney, but is increased by chemical treatments that result in cytolethality and regenerative cell proliferation.

Key results from this set of studies are that chloroform administered by gavage in corn oil generally resulted in increased labeling index in the liver and kidney of male and female B6C3F1 mice and F344 rats. The levels of the increases varied depending on the sex, species and strain of rodent used, as well as the duration of exposure. There was, however, a tendency for the degree of labeling to decline with the duration of dosing. An increased labeling index was generally not observed in the liver or kidney of female B6C3F1 mice or male F344 rats administered chloroform in drinking water. (Male mice and female rats were not tested using drinking water administration.) Following inhalation exposure to chloroform, a significantly increased labeling index was observed in the liver of male and female B6C3F1 mice and F344 rats after exposure for as long as 90 days. The kidney labeling index was also significantly increased in male and female B6C3F1 mice, and F344 rats. Recent studies investigated cell proliferation in additional strains used in chloroform bioassays. In male Osborne-Mendel rats (the strain and sex that form the basis for the chloroform cancer value derived in U.S. EPA, 1994, and in this document) administered a single gavage dose of chloroform, there was no increase in the liver labeling index, but there was a small increase in labeling index in the kidney; longer-term cell proliferation assays have not been conducted in this strain. In a 90-day cell proliferation assay conducted with male and female BDF_1 mice [the strain used in the inhalation bioassay of Matsushima (1994)], a significantly increased labeling index was observed in the kidneys of males, but not females.

A smaller increase in the labeling index was observed in the livers of males and females at 7 weeks, but the increase in males was not sustained through the end of the study.

Pereira (1994) reported that a small increase in the labeling index persisted for 159 days in the liver of female rats administered chloroform by corn oil gavage, with a LOEL of 263 mg/kg-day. This study observed no increase in labeling index in female rats administered chloroform in drinking water at doses up to 363 mg/kg-day for 5 to 159 days.

Chiu et al. (1996) compared the available data on short-term, subacute, and long-term cytotoxicity with the tumor incidences in different strains and species of the exposed rodents, as reported by published studies. Special attention was given to the subchronic (Jorgenson and Rushbrook, 1980) and chronic data of the Jorgenson et al. (1985) drinking water study, because it was the principal study used by EPA (1994) to derive the oral cancer risk estimate for chloroform. There were no treatment-related biochemical or microscopic/gross histopathological changes in the kidney of the rats after 30, 60, or 90 days of exposure to chloroform in drinking water. Chloroform carcinogenicity appeared to be associated with cytotoxicity in some cases, but not in others, depending on the strain and species of rodent tested. Data revealed that chloroform exposure, either by drinking water or by corn oil gavage, induced kidney cancer in male Osborne-Mendel rats without accompanying cytotoxicity at necropsy in the 2-year bioassays. In contrast, ILSI (1997) reported that, based on a rereading of the available slides from the male rats in the Jorgenson drinking water study, all high-dose rats exposed for 2 year had evidence of low grade chronic renal tubule injury and regeneration, where the tissues were not compromised by autolysis or diffuse diseases. Similar or minor lesions were observed in high-dose rats exposed for 18 or 12 months, and at 900 ppm, but not at 400 ppm. Chronic nephropathy was observed in all dose groups, including the controls, but the pathologist was able to distinguish the chronic nephropathy from evidence of cytotoxicity and regeneration.

Fox et al. (1990) used a mutational analysis of the *H-ras* oncogene in liver tumors in male C57BL/6 x C3H/HE mice (i.e., B6C3F1 mice) to address the issue of the mechanism of chloroform carcinogenicity. The spontaneous incidence of liver tumors in males of this strain is high (20-30%). Mutations activating the oncogene were found in 64% of the spontaneous tumors analyzed. By contrast, only 5/24 (21%) of the liver tumors from mice treated with chloroform (200 mg/kg by gavage in corn oil, twice weekly for 1 year) had activated *H-ras* genes. About 59% of the liver tumors found in mice treated with a known mutagenic carcinogen (benzidine hydrochloride) had an activated *H-ras* gene. Because the number of tumors with activated *H-ras* genes in the chloroform-treated group was not comparable to that expected spontaneously, the authors suggested that the observed tumors with an *H-ras* mutation occurred spontaneously, and the chloroform-induced liver tumors occurred via a different mechanism.

Sprankle et al. (1996) examined the levels of gene expression of various oncogenes in the liver of female B6C3F1 mice and kidneys of male F344 rats from the study of Larson et al. (1993). Mice

received a single oral gavage dose of 350 mg/kg chloroform in corn oil, and rats received a single oral gavage dose of 180 mg/kg chloroform in corn oil. There were transient increases in mRNA levels for the *myc* and *fos* growth control genes in the female mouse liver, but levels of *Ha-ras*, *met*, and hepatocyte growth factor mRNA were comparable to control levels. In the male rat kidney, there was a transient increase in levels of *myc* mRNA, but no strong effects on the other growth control genes examined. The study authors stated that other cytotoxic carcinogens induce a similar pattern of gene expression, and concluded that the changes in *myc* and *fos* expression could play a role in chloroform-induced regenerative cell proliferation.

Vorce and Goodman (1991) examined the methylation state of *ras* oncogenes in chloroform-induced liver tumors in male B6C3F1 mice. The mice were dosed by gavage twice weekly for 1 year with 200 mg/kg chloroform in corn oil. Liver tumors were reported in 80% of the animals, but the size of the treated group was not reported. The study authors found that *Ha-ras* was hypomethylated in all chemical-induced and spontaneous liver tumors examined, and sporadic hypomethylation of *Ki-ras* was observed in the spontaneous liver tumors, as well as chloroform-induced tumors. There was no effect on the methylation of *myc*, but there was some evidence of *myc* gene amplification. Because hypomethylation appears to be necessary, although not sufficient, for transcription, the study authors suggested that hypomethylation of *ras* genes may play a role in liver tumor development in this strain.

Dees and Travis (1994) found that chloroform exposure (0.5–2.0%, v/v) resulted in a slight hypermethylation of the p53 protein in both the RLE rat cell line, and in the human sarcoma line Saos-2 transfected with the gene for the tumor suppressor p53. Stronger hypermethylation was observed with the tumor promoter phorbol myristate acetate, and with benzene and toluene. Because the compounds investigated have been reported to stimulate protein kinase C, the study authors hypothesized that these compounds increase p53 methylation by stimulating protein kinase C. The authors suggested that this study and others investigating the possibility of protein kinase C-mediated tumor promotion by chloroform provide support for an alternative mechanism for chloroform carcinogenicity.

2.1.6 Potential Interactions

As discussed in U.S. EPA (1994), a variety of chemicals have been found to potentiate chloroform toxicity. Inducers of the forms of cytochrome P450 that metabolize chloroform result in increased toxicity, due to the increased production of reactive metabolites. Conversely, inhibitors or inactivators of P450 would decrease chloroform toxicity. Several ketones and chemicals that are metabolized to ketones also increase chloroform hepatotoxicity by a mechanism not solely related to enzyme induction. Mechanisms proposed for this potentiation include an effect on calcium pump activity and increased susceptibility of organelles. Suicide inactivators of CYP2E1, which are activated by the enzyme but bind covalently to it, include carbon tetrachloride, vinyl chloride, and trichloroethylene. The extent of all of these interactions, however, has not been well quantified.

The interaction between chloroform and dichloroacetic acid (DCA) or trichloroacetic acid (TCA) is of greater interest, since DCA and TCA are also drinking water disinfectant byproducts. (Both DCA and TCA are ketogenic compounds.) Davis and colleagues have conducted a series of experiments investigating these interactions (Davis, 1992; Yang and Davis, 1997a and 1997b). Pretreatment of fasted male and female Sprague-Dawley rats with three gavage doses of 2.45 mmol/kg/dose in 24 hours (total of 947 mg/kg) markedly increased the hepatotoxicity observed from a 3.12 mmol/kg (372 mg/kg) i.p. dose of chloroform. DCA alone was not hepatotoxic, and minimal to no effects were seen at this dose of chloroform alone. A larger effect was seen when rats were fasted prior to challenge with chloroform. The authors noted that fasting depletes protein stores, induces P4502E1 activity, and decreases hepatic glutathione content. In another study, nonfasted rats were administered DCA or TCA under the same conditions at 0.92 or 2.45 mmol/kg/dose, followed three hours later by a single i.p. dose of 75 mg/kg chloroform. Based on blood biochemistry, this chloroform dose caused hepatotoxicity and nephrotoxicity in females, but only marginal liver and kidney effects in males. DCA increased the chloroform hepatotoxicity and nephrotoxicity, and TCA increased chloroform nephrotoxicity, but interactive effects were seen only in females. *In vitro* data showed increased chloroform metabolism following DCA treatment. Although small but statistically significant increases in total cytochrome P450 levels and decreases in glutathione were observed *in vivo*, the authors noted that the increase in toxicity *in vivo* could not be attributed to increased cytochrome P450 levels, decreased hepatic glutathione, nor increased chloroform dose to the liver.

As noted for the examples of DCA and TCA, contaminants that interact with chloroform by would have the effect of shifting the dose-response curve for chloroform to the left. A large enough shift of the curve could move a given exposure toward the linear region of the dose-response curve for cytotoxicity, and hence carcinogenicity. Clearly such high exposures should be avoided.

In the low-dose range associated with environmental exposures, toxicity data on DCA and chloroform interactions are not available. Data on DCA exposure of humans is limited to information on drinking water levels. As reported by ILSI (1997), IARC (1995) reported that levels of DCA in drinking water are generally less than 100 ug/L, corresponding to a dose of approximately 0.003 mg/kg-day. As noted in Section 2.3, total mean chloroform intake via ingestion, inhalation, and dermal contact is approximately 0.002 mg/kg-day, with total intake of 0.01 mg/kg-day estimated for individuals consuming tap water containing relatively high levels of chloroform. As noted in the Technical Support Document on Risk Assessment of Chemical Mixtures (U.S. EPA, 1988), mechanistic considerations suggest that "additivity may be a plausible assumption in the low-dose region because thresholds for many types of interactions are expected to exist." Thus, it is reasonable to conclude, in the absence of data to the contrary, that DCA and chloroform interactions at low dose would be additive. This conclusion is further supported by recognizing that the public is exposed to more than just DCA and chloroform in drinking water, and that not all of these interactions will be synergistic. In fact, some of them are expected to be antagonistic.

2.2 Characterization of Dose Response

2.2.1 Quantification of Noncarcinogenic Effects

U.S. EPA (1994) developed Health Advisories and a Reference Dose (RfD) for the noncarcinogenic effects of chloroform. The basis for these values is discussed in the Drinking Water Criteria Document on Trihalomethanes (U.S. EPA, 1994) and on IRIS (U.S. EPA, 1998).

A One-day Health Advisory (HA) value for chloroform of 4 mg/L was calculated from an acute oral No-Observed-Adverse-Effect Level (NOAEL) of 35 mg/kg in mice. A NOAEL value of 35 mg/kg-day, identified in pregnant rabbits dosed by gavage on days 6 to 15 of gestation, was used to calculate a Ten-day HA value of 4 mg/L. No adequate data were located for calculating Longer-term HA values for chloroform, so the Drinking Water Equivalent Level (DWEL) of 0.4 mg/L (based on the RfD, see below) may be taken as a conservative Longer-term HA for adults, and the adjusted DWEL (0.1 mg/L) as a conservative Longer-term HA for children.

A Reference Dose (RfD) of 0.01 mg/kg-day was based on a Lowest-Observed-Adverse-Effect Level (LOAEL) for minimal liver injury (e.g., slightly elevated SGOT levels and an increased number of fatty cysts in the liver) of 15 mg/kg-day identified in a 7.5-year study in dogs (Heywood et al., 1979). The critical study was of chronic duration, used a fairly large number of dogs, and measured multiple endpoints; however, only two treatment doses were used and no NOAEL was determined. An uncertainty factor of 1000 was used; subfactors of 10 were judged appropriate for interspecies extrapolation, intraspecies variability, and LOAEL to NOAEL adjustment. U.S. EPA judged the confidence in the study as medium. Confidence in the data base was considered medium to low; several studies support the choice of a LOAEL, but a NOAEL was not found. Confidence in the RfD was also considered medium to low. After adjusting for an adult consuming 2 liters of tap water per day for a 70 kg adult, and applying a relative source contribution of 80% because most exposure is likely to come from drinking water, the MCLG is estimated to be:

MCLG Based on RfD for hepatotoxicity = 0.01 mg/kg-day x 70 kg x 0.8 / 2 L/day
= 0.3 mg/L(rounded)

2.2.2 Quantification of Carcinogenic Effects

Chloroform has been reported to be carcinogenic in several different chronic animal studies, increasing the frequency of liver tumors in male and female mice administered chloroform by gavage in oil, but not in female mice administered chloroform in drinking water, and increasing the incidence of kidney tumors in male rats and certain strains of male mice. The U.S. EPA (1985) reviewed the evidence on the carcinogenicity of chloroform and ranked it as a Group B2 carcinogen (probable human carcinogen). Because the formation of liver tumors in mice appears to be dependent upon the use of an oil vehicle, U.S. EPA (1987) recommended that the calculation of the cancer risk estimate for

chloroform be based on the incidence of renal tumors in male rats exposed to chloroform in drinking water from the Jorgenson et al. (1985) study. This is the current position on EPA's Integrated Risk Information System (U.S. EPA, 1998), and is the position of this document as well.

Using EPA's 1986 cancer guidelines, the potency of chloroform from this study is estimated to be 6.1×10^{-3} $(mg/kg\text{-}day)^{-1}$ by applying the LMS model. This results in a unit risk value of 1.7×10^{-7} $(ug/L)^{-1}$ and a corresponding drinking water concentration of 6 ug/l at an upper bound excess cancer risk level of 1×10^{-6}. Using the body weight to the 3/4 power conversion instead of the body weight to the 2/3 conversion, the potency calculated using the LMS model is 4.0×10^{-3} $(mg/kg\text{-}day)^{-1}$.

Using recommendations for linear extrapolation from the U.S. EPA's draft cancer guidelines (U.S. EPA, 1996), an ED_{10} and an LED_{10} of 37 and 23 mg/kg-day, respectively, were calculated in U.S. EPA (1997b) using the same data set.[4] Using the LED_{10} the resulting potency is 4.3×10^{-3} $(mg/kg\text{-}day)^{-1}$. The resulting unit risk value is 1.2×10^{-7} $(ug/L)^{-1}$ and the corresponding drinking water concentration is 8 ug/L at an upper limit excess cancer risk level of 1×10^{-6}. Use of the ED_{10} instead of the LED_{10} results in values that are approximately 60% higher.

In view of the weight of evidence that chloroform may induce tumors by a nonlinear mechanism (ILSI, 1997), a margin of exposure approach for dose-response analysis might be employed. U.S. EPA (1997b) calculated an ED_{10} of 37 mg/kg-day and an LED_{10} of 23 mg/kg-day) from tumors in the kidney of Osborne-Mendel rats administered chloroform in drinking water (Jorgenson et al., 1985). Dividing these by an estimated margin of exposure (MOE) can result in an exposure from which recommended water concentration may be derived.

U.S. EPA (1996) considers several areas of uncertainty that should be addressed with any MOE analysis. These areas are the slope of the dose response curve, the nature of the response modeled, the nature and extent of human variability, the persistence of the agent in the body, and the human sensitivity to the critical effect as compared with experimental animals. In the case of chloroform, the overall MOE might be as high as 1000, for these areas combined as discussed briefly below.

The use of a 10-fold default factor for intra-human variability is appropriate and could be recommended *in lieu* of specific data on differences in dynamics among individuals, and based on expected differences in the metabolism of chloroform due to differences in the CYP2E1 enzyme (e.g., Lucas et al., 1993; Stephans et al., 1994). The use of a additional 10-fold default factor for inter-species variation is appropriate and could be supported as per recommendation in the 1996 guidelines (U.S. EPA, 1996). The use of a final 10-fold factor is appropriate for the remaining uncertainties

[4] Except that values were determined using the adjustment of 3/4ths power of body weight between rats and humans, rather than the 2/3rds power of body weight currently on IRIS (U.S. EPA, 1998).

associated with the mode of carcinogenic action understanding, the slope of the dose response curve, and the lack of chloroform persistence in the body. For chloroform, the slope of the dose response curve for kidney tumors is shallow in comparison to curves for other chemicals, but not necessarily similar endpoints (Dourson and Stara, 1983).[5] Perhaps contrary to what one might expect, a shallower curve is expected to be associated with more need for margin of exposure. This is because a shallower slope means that the nonlinearity in the dose response curve is less quickly achieved as high dose is extrapolated to low dose (when compared to chemicals with steeper dose response curves). (In other words, for two chemicals with nonlinear modes of action and the same LED_{10}, the dose to the experimental animal below which the tumorigenic action would not be expected to occur would be lower for the chemical with shallower slope.) In contrast, the lack of persistence of chloroform in the body is supported by the known rapid clearance and excretion of chloroform when compared to other chemicals. Thus, lack of persistence indicates that more MOE is not needed. When these areas of uncertainty are folded together, an overall factor of 10 is reasonable.

Therefore, 37 or 23 mg/kg-day is divided by a MOE of 1000, giving 0.037 and 0.023 mg/kg-day, respectively. After adjusting for a 70 kg adult consuming 2 liters of tap water per day, and applying a relative source contribution of 80% (EPA assumes that drinking water is the predominant source of chloroform intake), the MCLG based on tumor responses is estimated to be:

MCLG for Chloroform (Based on LED_{10} for Tumor Response) =
0.023 mg/kg-day x 70 kg x 0.8/2 L/day = 0.6 mg/L (rounded)

MCLG for Chloroform (Based on ED_{10} for Tumor Response) =
0.037 mg/kg-day x 70 kg x 0.8/ 2 L/day = 1 mg/L (rounded)

Alternatively, an ED_{10} or LED_{10}, for the same endpoint, development of tumors in the kidney of Osborne-Mendel rats administered chloroform in drinking water (Jorgenson et al., 1985) could be expressed in terms of the tissue dose, rather than the administered dose. For example, ILSI (1997) proposed an ED_{10} or LED_{10} of 71 or 59 (respectively) mg/hr/liter of liver, based on the maximum rate of metabolism of chloroform to phosgene. If a human physiologically based pharmacokinetic (PBPK) model were available, such a model could be used to convert the ED_{10} or LED_{10} estimated by ILSI (1997) based on tissue dose to human equivalent doses. The construction of a human PBPK model is still needed; this is an area for future work.

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[5] The slope of the probit, log administered-dose response curve for the kidney tumors evoked by chloroform in the Jorgenson et al. (1985) study is approximately 0.6. An appropriate comparison of steepness in this dose response curve would perhaps be to cancer slopes from other chemicals. However, while numerous values of the "slope factor" q1* have been calculated, little information is available on the slope of cancer dose-response curves in the range of the experimental data. In the absence of such data, the lethality curves of Dourson and Stara (1983) were used for comparison.

It might also be appropriate to base the point of departure on cytotoxicity and regenerative cell proliferation endpoints, since the dose response for tumor incidence and increased cell proliferation were similar. The ILSI panel (ILSI, 1997) noted that the cytotoxicity data have been reported as means ±SD. In order to determine an ED_{10} or LED_{10} for cell proliferation, it would be necessary to define an increase in cell proliferation that would be considered to be adverse, and then determine the incidence of adverse responses based on individual animal data. In the absence of such data, tumor incidence was considered an appropriate endpoint for analysis in the observable range, in light of the similarity of the dose-response curves for the two endpoints.

Table 1 summarizes the quantification of noncarcinogenic and carcinogenic effects for chloroform.

Table 1. Summary of Quantification of Toxicological Effects for Chloroform

Health Advisory (HA), DWEL, Risk Specific Concentration (RSC) or MOE-based concentration	Concentration mg/L*
One-day HA for 10-kg child	4
Ten-day HA for 10-kg child	4
Longer-term HA for 10-kg child	0.1
Longer-term HA for 70-kg adult	0.4
DWEL based on RfD	0.4
MCLG based on RfD	0.3
U.S. EPA 1986 concentration at 10^{-6} risk level	0.006
U.S. EPA 1996 concentration at 10^{-6} risk level	0.008
MCLG based on MOE from tumor endpoint at LED_{10} & exposure dose	0.6
MCLG based on MOE from tumor endpoint at LED_{10} & tissue dose	future effort
MCLG based on MOE from cytotoxicity at LED_{10} and tissue dose	future effort

*Estimates of concentrations incorporate consumption assumptions of 2 liters of water per day and 70 kg body weight for adults, and 1 liter of water per day and 10 kg child body weight. The MCLG concentrations further include an 80% relative source contribution of drinking water to the overall chloroform exposure.

2.3 Characterization of Exposure

U.S. EPA (1994, 1997a) summarized information on human exposure to chloroform. Information below has been extracted from this document, unless otherwise noted.

Chloroform is found in virtually all treated drinking water; however, concentrations vary widely depending on the type of water treatment, locale, time of year, and source of the drinking water. Chloroform concentrations in drinking water have ranged from less than 0.5 to 550 ug/L (ppb). Concentrations of all chloroform in drinking water is generally lower when the raw water is derived from ground water sources rather than surface water sources.

Chloroform has been detected in food at concentrations ranging from non-detectable to 830 ng/g (ppb). Chloroform is approved by FDA as an indirect food additive for use as a component of adhesives or polycarbonate resins used in food packaging.

Chloroform is ubiquitous in air, although the concentrations are highly variable depending on the ambient environment. Chloroform concentrations tend to be higher in indoor air compared to outdoor air because of the confined space and release of chloroform from various indoor sources. Chloroform concentrations in personal air and outdoor air ranged from 0.06 to 215 ug/m^3 (0.01–44 ppb) , and from 0.04 to 21.5 ug/m^3 (0.008–4.4 ppb), respectively. One major source of chloroform in indoor air appears to be from tap water that releases chloroform when used for showers or washing. One study indicated that concentrations of chloroform in shower stall air samples during a 10-minute shower ranged from 10 to 500 ug/m^3 (2.05–112 ppb). The absorbed inhalation and dermal doses were 0.24 and 0.23 ug/kg/day, respectively, for a combined absorbed chloroform dose from a 10-minute shower of 0.47 ug/kg/day.

The use of chlorine to disinfect swimming pools and hot tubs also results in the release of chloroform to the overlying air. One study indicated that chloroform concentrations in swimming pool and hot tub water ranged from less than 1 to 530 ug/L. Concentrations of chloroform in the air two meters above the pool water ranged from 0.1 to 260 ug/m^3 (0.2–53 ppb).

Chloroform exhaled in breath is related to body burden of chloroform and recent exposure to chloroform in air or water. Background chloroform concentrations measured in breath have ranged from 0.22 to 5.06 ug/m^3 (0.05–1.04 ppb), and reported breath concentrations after a 10-minute shower ranged from 6 to 21 ug/m^3 (1.23–4.3 ppb). Chloroform has also been detected in the blood, milk and adipose tissue of humans. Chloroform concentrations in blood have ranged from less than 0.1 to greater than 25 ug/L (ppb). Chloroform has been detected in the milk of 7 of 49 lactating women living in industrial areas; however, actual concentrations were not reported.

A number of authors have calculated inhalation and ingestion rates based on various assumptions. ILSI (1997) summarizes some of these estimates and provides some of their own which are reported here.

ILSI (1997) estimated mean intake of chloroform from indoor air for the general population to be 0.3 to 1.2 ug/kg body weight per day. This is based on a daily inhalation volume for adults of 22 m^3, a mean body weight for males and females of 64 kg, the assumption that 20 out of 24 hr are spent indoors (IPCS, 1994), and mean levels of chloroform in indoor air (1 to 4 ug/m^3).

As stated by ILSI (1997), individuals may be exposed to elevated concentrations of chloroform (from chlorinated tap water) during showering (Jo et al., 1990a, 1990b). Based on assumptions of an absorption efficiency from the respiratory tract of 0.77, a breathing rate of 0.014 m^3/min for a 70-kg adult, a measured mean concentration in shower air of 157 ug chloroform/m^3 and a ratio of body burden resulting from dermal exposure to that of inhalation exposure of 0.93, these authors estimated that the average intake of chloroform (inhalation and dermal absorption) was 0.5 ug/kg body weight per shower for a person weighing 70 kg.

ILSI (1997) stated that based on a review of relevant estimates, Maxwell et al. (1991) concluded that the ratio of the dose of chloroform received over a lifetime from inhalation to that received from ingestion of drinking water is probably in the range of 0.6-1.5 but could be as high as 5.7. The ratio of the dose received dermally to that received orally over a lifetime from drinking water was considered to be approximately 0.3 but could be as high as 1.8.

ILSI (1997) estimated mean intake of chloroform from drinking water for the general population is less than 0.5 ug/kg body weight per day. This is based on a daily volume of ingestion for adults of 1.4 liters and a mean body weight for males and females of 64 kg (IPCS, 1994), with mean levels of chloroform in drinking water (generally < 20 ug/liter). As discussed by Bauer (1981), actual levels of exposure may be less than those estimated on the basis of mean levels in drinking water since most of the chloroform would be expelled from drinking water that is heated before consumption (tea, coffee, soups, sauces). For example, approximately 96% of the total volatile halogenated hydrocarbon fraction was eliminated in water boiling for 5 min, whereas 50-90% was eliminated upon heating at 70-90 ^oC (Bauer, 1981). (Halogenated hydrocarbons eliminated in this way could of course be inhaled.) It should be noted, however, that owing to the wide variations in concentrations of chloroform in water supplies, intake from drinking water could be considerably greater than estimated here for some segments of the general population.

ILSI (1997) estimated daily intake of chloroform from foodstuffs to be approximately 1 ug/kg body weight per day. This is based on a daily volume of ingestion of solid foodstuffs for reference adults of 1.5 kg and a mean body weight for males and females of 64 kg (IPCS, 1994), and the mean level and percentage detection of chloroform in foodstuffs in the market-basket survey reported by Daft (1989).

As stated also by ILSI (1997), based on estimates of mean exposure from various media, therefore, the general population is exposed to chloroform principally in food, drinking water, and indoor air in approximately equivalent amounts. The estimated intake from outdoor air is considerably less. The total estimated mean intake is approximately 2 ug/kg body weight per day. For some individuals living in dwellings supplied with tap water containing relatively high concentrations of chloroform, estimated total intakes from drinking water through ingestion, inhalation, and dermal contact are up to 10 ug/kg body weight per day. These multi-media exposure estimates can be used as combined route inputs with a human PBPK model to estimate metabolized dose in target tissues.

3. Risk Conclusions and Comparisons

3.1 Key Lines of Evidence for Critical Effect

3.1.1 Overall Conclusion

Chloroform causes the development of cancer in several animal species by a mechanism that is believed to be non-linear in the low-dose range. Chloroform will likely be carcinogenic to humans by all routes of exposure if a sufficient dose is administered. Human data are insufficient to determine chloroform's potential carcinogenicity. Experimental animal studies are somewhat variable in both tumors evoked and magnitude of response. The principal studies for the determination of chloroform's tumorigenic effect and low dose extrapolation are NCI (1976), in which chloroform was administered by gavage in oil to male and Osborne-Mendel rats and B6C3F1 mice, and Jorgenson et al. (1985), in which chloroform was administered in drinking water to male Osborne-Mendel rats and female B6C3F1 mice.

For noncancer toxicity a Reference Dose (RfD) was based on a Lowest-Observed-Adverse-Effect Level (LOAEL) for minimal liver injury of 15 mg/kg-day identified in a 7.5-year study in dogs (Heywood et al., 1979). The critical study was of chronic duration, used a fairly large number of dogs, and measured multiple endpoints. Confidence in critical study is considered medium, and in its supporting data base is considered medium to low. Overall confidence in the RfD is also considered medium to low, meaning that additional data may more likely change the value of the RfD when compared to a high confidence RfD (U.S. EPA, 1998).

3.1.2 Strengths and Weaknesses of the Evidence

As summarized in part by ILSI (1997), chloroform produces tumor responses in the liver and kidney, but the responses vary by route of exposure, sex and strain. In the liver, chloroform causes an increase in tumors only with corn oil gavage administration; other routes of exposure at similar or higher doses failed to induce a carcinogenic response in the liver. Chloroform also induces renal neoplasia in rats and mice, but this response is limited to males in both species. Renal tumors were found in two strains of mice (BDF1 and ICI), while other strains failed to show a renal tumor response. This strain-

specific difference appears to be related to a higher metabolic capability for chloroform bioactivation, and thus greater cytotoxicity, in the sensitive strains. In rats, renal tumors were found in the Osborne-Mendel strain. Studies in Wistar, Sprague-Dawley and F344 rats were negative for kidney tumors.

Several studies in animals support the conclusion that chloroform causes cancer after oral exposure, and at least two studies are strong enough to support quantitative dose response assessment (NCI, 1976; and Jorgenson et al., 1985). Of these two, the Jorgenson et al. (1985) study uses drinking water, which is preferred route and vehicle of administration. By contrast, the NCI (1976) bioassay used oil gavage; interpretation of the tumor response data from this study is complicated by the potential effect of the bolus dosing, and the potential effect of the oil on toxicity. One unpublished animal study is available that indicates tumor response after inhalation exposure (Matsushima, 1994). As noted above, however, the tumorigenicity of chloroform varies with the dosing regimen (e.g., drinking water versus gavage in oil), species, strain, and sex. The available information in humans is uninformative with respect to the potential carcinogenicity of chloroform.

3.1.3 Weight of Evidence: Key Conclusions, Assumptions and Defaults

Chloroform is likely to cause tumors in humans by multiple routes of exposure. The tumor-causing potential of chloroform has been demonstrated in both rats and in mice. The very weak or absent mutagenic activity of chloroform, however, suggests that the pathway to tumor formation involves an indirect production (or promotion) of mutations. Careful analysis of chloroform toxicokinetics and chloroform-induced pathology indicates that the observed tumors result from the oxidative metabolism of chloroform to phosgene and hydrochloric acid. These metabolites are cytotoxic in liver and kidney where they are produced at relatively high rates. Recurrent and continuing hyperplasia following these episodes of toxicity appears to be a necessary precursor for hepatic and renal tumor formation in rodents. It should be noted, however, that cell proliferation is a necessary, but not a sufficient condition for the development of chloroform-induced tumors.

The evidence for this mechanism is strong for liver tumors and for kidney tumors in mice. It is less strong for kidney tumors in rats, primarily due to the lack of data on cell proliferation in Osborne-Mendel rats following long-term exposure. One key assumption behind this conclusion is that the metabolism of chloroform in humans is similar to that in rodents for the target organs of interest. The data generally support this assumption qualitatively, based on similarities in the two main paths of metabolism, but notable quantitative differences exist among animal species in the amounts of metabolites formed. Another key assumption in this analysis is that the rate of cell killing and regrowth found in rodents would be similar to that in humans. This assumption is reasonable, based on our understanding of the toxicity of chloroform, and based on the similarity in target organs observed in the limited number of human case studies to those seen in animals. Another assumption is that the use of a rodent model *in lieu* of human data is a reasonable default. Much work in toxicology supports this procedure.

Another potential mode of action for the induction of tumors by chloroform might be through metabolism in the absence of oxygen (i.e., a reductive pathway), depletion of cellular defense mechanisms (e.g., glutathione), and DNA or other macromolecular damage. This mode of action does not appear likely *in vivo*, except at high doses or in induced animals. If present, it is likely to be quantitatively less extensive than the cytotoxic mode of action. It may also be, however, that chloroform causes tumor formation by both modes of action. The reductive pathway might lead to a small amount of DNA damage, and the cytotoxicity and regrowth prompted by the oxidative pathway might stimulate both naturally occurring mutations and chloroform-induced DNA damage.

3.1.4 Significant Issues and Uncertainties

As summarized in part by ILSI (1997), there are some data deficiencies; the remaining uncertainties for assessing chloroform are in defining the relationship of tissue metabolism to toxicity, and in assessing the pharmacokinetic parameters for chloroform metabolism in humans. Acquisition of additional information in these areas would provide greater confidence in the conclusion that tumors associated with exposure to chloroform are primarily a secondary consequence of marked cytotoxicity in direct association with a period of sustained cell proliferation induced by a metabolite.

The weight of evidence for an obligatory role of cytotoxicity in chloroform carcinogenicity is strongest for hepatic tumors in rats and mice, and for renal tumors in mice. The evidence is more limited for renal tumors in rats, primarily due to the relative paucity of data on intermediate endpoints (e.g., cell proliferation) in the strains where tumors have been observed. Uncertainty could be reduced by acquisition of additional information on cytotoxicity and proliferative response in the strain in which tumors were observed (i.e., Osborne-Mendel rats) following long-term exposure to chloroform, Additional data on chronic (e.g, 2-year) cytotoxicity/proliferative response in the kidneys of F344 rats might also contribute to greater confidence in the hypothesized mode(s) of action.

Although the weight of evidence supports the claim that chloroform is not mutagenic, one area which could be clarified by further work is whether any of the metabolites of chloroform are DNA-reactive. Data available for phosgene indicate that, while it is extremely reactive, it is likely to bind to other cellular components prior to reaching the genetic material in the cell nucleus. No studies on the genotoxicity of phosgene were located.

It would also be desirable to clarify whether the same pathways of metabolism contribute to the potential for cytotoxicity in rodents and humans, specifically with respect to CYP2E1. In mice, it is clear that this pathway is responsible for much (if not all) of the cytotoxic responses in liver and kidney. CYP2E1 activity in mice and rats has been localized to the centrilobular region of the liver and to the cortex of the kidney. It is not known whether other P450 isoenzymes contribute to the metabolism of chloroform in humans. Data on the localization and levels of CYP2E1 in potential target organs in humans are not available.

If the development of MOE is to be based on tissue doses, then some uncertainty exists in the choice of the appropriate tissue dose surrogate for the putative toxic metabolite of chloroform (i.e., phosgene) as made by ILSI (1997). ILSI (1997) also did not attempt to assess pharmacokinetic parameters (tissue metabolism rates and the size of the regions of the tissues which have chloroform metabolizing enzymes) for chloroform metabolism in humans. These parameters need to be evaluated before extending the rodent PBPK model to humans.

3.1.5 Alternative Conclusions

Metabolism of chloroform in the absence of oxygen (i.e., reductive metabolism) will lead to the production of free radicals. One of the body's normal protections against free radicals is the naturally occurring chemical glutathione. If glutathione is depleted by excess production of free radicals, this may in turn lead to genotoxicity, which may be responsible for chloroform's tumor production. However, this pathway is recognized to be a minor one at most, and would likely be of relevance only at high doses or in induced animals.

3.2 Likelihood of Human Harm

Sensitive subgroups of humans to chloroform's toxicity have not been identified. However, people with increased levels of chloroform metabolism, such as resulting from alcohol-related CYP2E1 induction, might be expected to have an increased susceptibility to the effects of chloroform. People with pre-existing liver or kidney dysfunction would also be expected to be more sensitive to the effects of chloroform, since they would have a decreased functional reserve. Based on a comparison of metabolic capacity and sensitivity to systemic effects in fetal and young rats versus adult rats, and based on the doses at which systemic effects have been seen in adult rats versus developmental effects in rats and rabbits, children and fetuses do not constitute a sensitive population.

The margin of exposure approach taken for chloroform is considered to be protective of susceptible groups, including children. The mode of action understanding for chloroform's carcinogenic and cytotoxic effects is not age related and involves a generalize mechanism of toxicity that is seen consistently across different species. Furthermore, CYP2E1 generated metabolites are key to chloroform's mode of action, and CYP2E1 activity is lower in children compared to adults or at least not more than adults (Casazza et al., 1994). Additionally, the margin of exposure analysis is designed to be protective for sensitive populations. In the case of chloroform, an additional 10 factor was used to account for variability between the average human response and the response of more sensitive individuals. Note that the use of this factor presupposes that the variation in human response is more than 10-fold, since it accounts for differences in average to sensitive, and not resistant to sensitive individuals.

The average human exposure to chloroform from air, water and food is approximately 2 ug/kg/day (ILSI, 1997). This average exposure will not likely yield the occurrence of tumors in humans

because it is about 12 to 19-fold less than the estimated MOE-based dose of 23 or 37 (based on either the LED_{10} or ED_{10}, respectively) ug/kg/day, which is derived from the tumor response in rat kidney. Noncancer toxicity is not expected at this existing chloroform exposure either, since this combined exposure of 2 ug/kg/day is approximately 5-fold below the existing RfD of 10 ug/kg/day.

Certain subgroups of humans may be at greater risk from chloroform toxicity because of exposures that exceed the average daily intake of ~2 ug/kg-day. For example, some individuals living in dwellings supplied with tap water containing relatively high concentrations of chloroform have estimated total intakes from drinking water through ingestion, inhalation, and dermal contact of up to 10 ug/kg body weight per day (ILSI, 1997). These high-end total exposures via the ingestion, inhalation, and dermal routes are of the same magnitude as the RfD of 10 ug/kg/day. However, even at these higher exposures, neither the RfD nor the MOE-based dose are exceeded.

3.3 Dose Response Assessment/Characterization

3.3.1 Overall Conclusions

Mechanistic data are not sufficiently developed in order to generate a biologically based, or case specific, model for chloroform's toxicity. However, a sufficient amount of data are available to postulate a possible mode of action. In such cases, a default linear or nonlinear approach (or both) is recommended for the dose response assessment depending on the available data (U.S. EPA, 1996). In view of the weight of evidence that chloroform may induce tumors by a nonlinear mechanism, a margin of exposure approach is reasonable for a dose-response analysis. Division of either the ED_{10} or the LED_{10} of 37 or 23 mg/kg-day estimated by U.S. EPA (1997b) from tumors in the kidney of Osborne-Mendel rats (Jorgenson et al., 1985) by an estimated margin of exposure (MOE) of 1000-fold, and using standard assumptions of body weight (70 kg), water consumption (2 L/day) and Relative Source Contribution (0.8), results in a MCLG of either 1 or 0.6 mg/L [i.e., 37 or 23 mg/kg-day ÷ 1000 * 70 kg * 0.8 (RSC) ÷ 2 L/d = 1 or 0.6 mg/L], respectively. See Figure 1.

Alternatively, the ED_{10} of 37 mg/kg-day (or the LED_{10} of 23 mg/kg-day) could be divided by the estimated average exposure of ~2 ug/kg-day (ILSI, 1997). If the ED_{10} were used, the resulting MOE would be ~19,000, meaning that the estimated average chloroform exposures in humans are ~19,000-fold below those doses which have been estimated to cause a 10% increase in tumors in rodents.

For noncancer toxicity an RfD of 0.01 mg/kg-day was based on a LOAEL for minimal liver injury in dogs (Heywood et al., 1979). Confidence in the critical study is considered medium, and confidence in the supporting data base is considered medium to low, resulting in overall medium to low confidence in the RfD (U.S. EPA, 1998).

3.3.2 Strengths and Weaknesses of the Data Available for Analysis

Several animal studies support the conclusion that chloroform causes cancer in animals. The assumption that it is likely to be carcinogenic in humans is reasonable. Differences among the animal studies can be explained in part by differences in type of exposure (single large daily doses versus more continuous consumption over the course of the day), or by differences in metabolism. Humans develop some of the same toxic responses to chloroform as do experimental animals after acute exposures, but the human data cannot be used to determine directly whether chloroform causes tumors in humans. Based on the weight of evidence, sufficient mode of action information is available to support a nonlinear default dose response assessment. Although some data may suggest that a linear default dose response assessment is appropriate, this approach is not supported by the overall weight of evidence.

The use of tumor data as a basis for the development of an ED_{10} and LED_{10} is traditional and well supported. Although use of tumor precursor data (e.g., cell proliferation) was addressed in the proposed cancer guidelines (U.S. EPA, 1996), sufficient data for determining an ED_{10} or LED_{10} for cell proliferation are not available. Use of the ED_{10} or LED_{10} based on tissue dose would improve the accuracy of the animal to human extrapolation. However, pharmacokinetic calculations of human tissue doses have not been performed, although the development of such calculations appears possible given the available data on enzyme levels in human liver and kidney.

3.3.3 Selection of Study, Issues of Route, Frequency & Duration of Exposure

A traditional approach to the estimation of human dose from animal dose was conducted (U.S. EPA, 1997b) on the Jorgenson et. al. (1985) study, in which both benign and malignant kidney tumors were evoked by oral exposure to chloroform in drinking water. This calculation entails the estimation of the human dose in mg/kg-day by multiplying the animal dose by a 1/4 power of body weight ratio between animals and humans (often referred to as a body weight to the 3/4 power scaling). The use of this traditional approach allows easier comparisons with other carcinogens. Unfortunately, it does not use all of the available mode of action information on chloroform.

An alternative and perhaps preferred conversion of animal dose to human dose could be based on PBPK model. Such a model could be used to calculate estimates of the target tissue doses of phosgene, a primary chloroform metabolite, in those regions of the kidney or liver with high levels of chloroform metabolizing enzymes. A PBPK analysis has been performed to develop tissue doses of phosgene for rats (ILSI, 1997). Such analyses for humans would be needed before interspecies comparisons and low dose extrapolation could be made.

The likely chloroform exposure to the general population will be in food, drinking water and indoor air. Taken together, this combined exposure is more likely to be continuous rather than episodic, and more likely to be long-term rather than short-term. Single or episodic exposures may occur in the environment due to releases, or perhaps in the workplace due to spills. Such exposures,

associated risks and appropriate management solutions are not discussed here.

The most appropriate information on which to base a risk characterization of chloroform to the general population is from the extensive experimental animal data base, as briefly summarized in this document and more extensively elsewhere (U.S. EPA, 1994; U.S. EPA 1997a; and ILSI, 1997). Human data on the toxicity of chloroform are always more desirable than animal toxicity studies. However, from the narrow viewpoint of relevance of risk assessment, such data are not available in sufficient quantities or quality to yield credible assessments. The human data also can not at present be used to judge the appropriateness of the estimates of risk from the experimental animals.

The experimental animal studies on which the dose response assessments are based are from long-term exposures and continuous dosing (Jorgenson et al., 1985; Heywood et al., 1979). These two studies paid careful attention to determining the doses associated with effects and targets of chloroform (i.e., in the liver and kidney) identified by other animal studies and in the limited available human data. The exposure route in at least one of these studies (drinking water, in Jorgenson et al., 1985) matches the expected human exposure routes more closely than other rodent studies that used oil gavage, thereby increasing the confidence in the resulting estimates of risk.

3.3.4 Strengths & Weaknesses of the Assessment: Issues & Uncertainties

The weight of evidence suggests that chloroform induces tumors by a nonlinear secondary mechanism and that a nonlinear dose response is reasonable. As per EPA proposed guidelines (1996), a margin of exposure approach is therefore recommended. The basis of this MOE is either the ED_{10} of 37 mg/kg-day, or the LED_{10} of 23 mg/kg-day, as estimated by EPA (1997b) from tumors in the kidney of Osborne-Mendel rats exposed to chloroform in drinking water (Jorgenson et al., 1985). Division of either the ED_{10} or the LED_{10} by an estimated margin of exposure (MOE) of 1000 yields doses in the approximate range of 30 ug/kg-day.

The weight of evidence for the mode of action of chloroform supports the expected nonlinear behavior. For example, cytotoxicity and cell regrowth is nonlinear (ILSI, 1997). Moreover, the assumption of a linear dose response assessment for carcinogens is often predicated on the strength of their mutagenicity, and in this regard chloroform is equivocally negative.

Alternative measures of ED_{10} or the LED_{10} have been proposed (ILSI, 1997). For example, the estimation of an ED_{10} of 71.3 mg/liter of kidney cortex/hour for increased incidence of kidney tumors in rats is well supported by the consistency of the dose response data under other dosing protocols and in other species. Additional data on cell proliferation in rats would be needed to strengthen this alternative assessment, since proliferation is a key parameter in its use. A margin-of-exposure of less than 1000 fold might be applied to this latter ED_{10} because the use of a PBPK model could reduce some of the uncertainties in the rat-to-human extrapolation.

The choice of a nonlinear approach to the dose response assessment is reasonable given the weight of the overall evidence for the induction of tumors by chloroform in rodents. The assumption that such tumors would be evoked in humans by a similar mode of action, given a sufficient dose, is also reasonable, based on our understanding of the metabolism of chloroform by mammals in general, and the types of effects evoked by chloroform in rats and humans specifically. Metabolism does vary among species, however, which introduces some uncertainty into the extrapolation from rats to humans.

Alternative modes of action are theoretically possible, for example, tumors may be evoked by gene mutations from free radicals via reductive metabolism after cellular defense mechanisms, such as glutathione, are depleted. However, at best the contribution of the reductive pathway to chloroform metabolism is quantitative much smaller than that of the oxidative pathway (ILSI, 1997).

It also may be that tumors are being induced by both modes of action, i.e., gene mutations by way of minor (at best) reductive metabolism and cytotoxicity and regrowth by oxidative metabolism. This latter supposition should be carefully explored in future research.

3.3.5 Basis of Assumptions and Defaults

Sufficient data are available on chloroform's kinetics and dynamics in order to postulate a default nonlinear approach to dose response assessment as per the proposed EPA guidelines (1996). Standard assumptions in this default dose response assessment include:

- the use of experimental animal data as a surrogate for humans,
- the use of kidney tumor response in rats (for cancer effects) and liver disease in dogs (for noncancer effects) as meaningful for extrapolating to human disease,
- the conversion of experimental doses in either rats or dogs to humans by either a 3/4ths power of body weight adjustment or division by a 10-fold uncertainty factor,
- the use of factors based on a logarithmic scale (10, 3 or 1) with either the MOE or RfD that address additional scientific uncertainties in the overall data base,
- the use of one digit of arithmetic precision for the MCLGs and DWEL because our understanding of the underlying biology is unlikely to be more precise than this.

The use of these and similar assumptions is common practice in conducting dose response assessments by other environmental and health agencies throughout the world.

3.3.6 Alternative Approaches

A default linear approach (also described in the proposed EPA 1996 guidelines) could be suggested for comparison to the nonlinear approach recommended here. The basis for this linear approach can be found in the traditional approach to the dose response assessment of carcinogens, and

is suggested by some because of the expected formation of free radicals during the reductive metabolism of chloroform (as discussed above). However, cellular defense mechanisms, such as glutathione, must be depleted before this formation of free radicals can occur, and the weight of evidence suggests that the reductive metabolism is quantitatively minor when compared to oxidative metabolism. Comparison with the current assessment on EPA's Integrated Risk Information System (IRIS) is also possible. Comparisons of the resulting water concentrations were presented above in Table 1. Figure 1 also shows the alternative approach.

3.4 Risk Characterization Summary

In the 1994 proposed rule, EPA classified chloroform under the 1986 EPA Guidelines for Carcinogen Risk Assessment as a Group B2; probable human carcinogen. This classification was primarily based on sufficient evidence of carcinogenicity in animals. Kidney tumor data in male Osborne-Mendel rats reported by Jorgenson et al. (1985) was used to estimate the carcinogenic risk. An MCLG of zero was proposed. Because the mode of carcinogenic action was not understood at that time, EPA used the linearized multistage model and derived a carcinogenicity potency factor for chloroform of 6×10^{-3} $(mg/kg\text{-}day)^{-1}$. The 95% upper bound limit lifetime cancer risk levels of 10^{-6}, 10^{-5}, and 10^{-4} were determined to be associated with concentrations of chloroform in drinking water of 6, 60, and 600 ug/L. Since the 1994 rule, several new studies are available providing insight into the mode of carcinogenic action for chloroform. EPA has reassessed the cancer risk associated with chloroform exposure (U.S. EPA, 1997b) by applying the principles of the 1996 EPA Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), which are considered scientifically consistent with the Agency's 1986 guidelines (U.S. EPA, 1986).

The recent report by ILSI (1997) was fully considered as well as the new science that has emerged on chloroform since the 1994 proposed rule. Based on this new information, chloroform is considered to be a likely human carcinogen by all routes of exposure. Chloroform's carcinogenic potential is indicated by animal tumor evidence (induced liver tumors in mice and induced renal tumors in both mice and rats) from inhalation and oral exposures, as well as metabolism, toxicity, mutagenicity and cellular proliferation data that contributes to an understanding of its mode of carcinogenic action. Although the precise mechanism of chloroform carcinogenicity has not been established, the ILSI (1997) report is a reasonable scientific basis to support a putative mode of carcinogenic action involving cytotoxicity produced by the oxidative generation of highly reactive metabolites (phosgene and hydrochloric acid), followed by regenerative cell proliferation as the predominant influence of chloroform on the carcinogenic process. This supports a nonlinear approach to extrapolating low dose risk. The ILSI (1997) report also discusses uncertainties associated with the chloroform assessment, which include lack of data on cytotoxicity and cell proliferation responses in Osborne-Mendel rats, mutagenicity data on chloroform metabolites, and the lack of comparative metabolic data in humans. These data deficiencies raise some uncertainty about how chloroform may influence tumor development at low doses.

Therefore, both a linear and nonlinear default approaches is applied to estimate cancer risk associated with drinking water exposure to chloroform. The nonlinear default or margin of exposure approach should be used in quantifying the cancer risk associated with chloroform exposure because the evidence is more compelling for a nonlinear mode of carcinogenic action. The linear dose-response extrapolation approach appears overly conservative in estimating low-dose risk, but nevertheless it is shown to account for remaining uncertainties. [It should be noted that the 1996 linear LED_{10} approach and the 1986 LMS approach resulted in similar unit risk estimations, 1.2 x 10-7 $(ug/L)^{-1}$ and 1.7 x 10-7 $(ug/L)^{-1}$, respectively].

The tumor kidney response data in Osborne-Mendel rats from Jorgenson et al. (1985) are used to serve as the basis for the point of departure because a relevant route of human exposure (i.e., drinking water) and multiple dose of chloroform (i.e., 5 doses including zero) were used in this study. The ED_{10} and LED_{10} for kidney tumors in this study were estimated to be 37 and 23 mg/kg-day, respectively (U.S. EPA, 1997b). These values were adjusted to equivalent human doses using the body weight to the 3/4 interspecies scaling factor, as proposed in the 1996 EPA cancer guidelines (U.S. EPA, 1996). Consistent with the EPA 1996 proposed cancer guidelines, a 100-fold factor was applied to account for the variability and uncertainty associated with intra- and interspecies differences, in the absence of data specific to chloroform. A science policy decision was made to apply an additional factor of 10 to account for the remaining uncertainties associated with the mode of carcinogenic action understanding, and the nature of the tumor dose response relationship being relatively shallow. The total factor of 1000-fold represents an adequate margin of exposure that addresses inter-and intra-species differences and uncertainties in the database. Other factors considered in determining the adequacy of the margin of exposure include the size of the population exposed, duration and magnitude of exposure, and persistence in the environment. Taking these factors into consideration, a MOE of 1000 is still regarded as adequate. Although a large population is chronically exposed to chlorinated drinking water, chloroform is not bio-persistent and humans are exposed to very low levels of chloroform in the drinking water, below those exposures needed to induce a cytotoxic response. (Levels of occurrence are typically as high as ~130 ug/L and median values are ~ 20 ug/L.) Therefore, 37 or 23 mg/kg-day is divided by a MOE of 1000, giving 0.037 and 0.023 mg/kg-day, respectively. After adjusting for a 70 kg adult consuming 2 L of tap water per day, and applying a relative source contribution of 80% (assuming that drinking water is the predominant source of chloroform intake), the MCLG based on tumor responses is estimated to be 0.6 mg/L based on the LED_{10}, or 1 mg/L based on the ED_{10}.

In the 1994 proposed rule, data from a chronic oral study in dogs were used to derive the RfD of 0.01 mg/kg/d (U.S. EPA, 1994). This RfD is based on a LOAEL for hepatotoxicity and application of an uncertainty factor of 1000. After adjusting for an adult consuming 2 L of tap water per day for a 70 kg adult, and applying a relative source contribution of 80% because most exposure is likely to come from drinking water, the MCLG is estimated to be:

MCLG Based on RfD for Hepatotoxicity =

0.01 mg/kg-day x 70 kg x 0.8 / 2L/day = 0.3 mg/L (rounded)

A MCLG of 0.3 mg/L based on hepatotoxicity (U.S. EPA, 1994) is more sensitive than the values determined from the LED_{10} /ED_{10} approach for kidney tumorigenesis (0.6 mg/L or 1 mg/L), and is consistent with chloroform's putative mode of action involving the oxidative generation of reactive and toxic metabolites (phosgene and hydrochloric acid). The exact mechanism by which these metabolites cause cytotoxicity is not known, but plausible mechanisms can be hypothesized based on an understanding of important nucleophilic targets in the cell. The electrophilic metabolite phosgene could react with phosphotidyl inositols or tyrosine kinases, which in turn could potentially lead to interference with signal transduction pathways thus leading to carcinogenesis. Glutathione, free cysteine, histidine, methionine, and tyrosine are all potential reactants for electrophilic agents. Likewise, it is also plausible that phosgene reacts with cellular phospholipids, peptides, and proteins, resulting in generalized tissue injury. Hepatotoxicity is the primary effect observed following chloroform exposure, and among tissues studied for chloroform-oxidative metabolism, the liver was found to be the most active (ILSI, 1997). It should be noted that the MCLG based on the RfD for hepatotoxicity, 0.3 mg/L, and the MCLG based on the ED_{10} for renal tumorigenesis, 1 mg/L, falls within the 5×10^{-5} to ~2×10^{-4} range predicted for cancer risk in the 1994 proposal using the LMS model. An MCLG based on protection against cytotoxicity should be protective against carcinogenicity given that the putative mode of action understanding for chloroform involves cytotoxicity as a key event preceding tumor development. Therefore, the recommended MCLG for chloroform is 0.3 mg/L or 300 ug/L.

Therefore, based on the available evidence, a nonlinear approach is considered for estimating the carcinogenic risk associated with lifetime exposure to chloroform via drinking water. The 1994 proposed MCLG was zero for chloroform; it is now considered to be 300 ug/L. Given that hepatic injury is a primarily effect following chloroform exposure, which is consistent with the mode of action understanding, the 1994 RfD based on hepatotoxicity is considered a reasonable basis for the chloroform MCLG. It should be noted that 300 ug/L equates to 5×10^{-5} cancer risk level using the LMS model for kidney tumors.

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APPENDIX: SUMMARY OF REVIEWERS' COMMENTS

Charge to External Peer Reviewers

Statement of Work

TITLE: Peer review of Health Risk Characterization Report on Chloroform

BACKGROUND:

The mission of the United States Environmental Protection Agency's (EPA) Office of Water (OW) is to protect public health and the environment from adverse effects of contaminants in media such as ambient water, drinking water, waste water, sewage sludge and sediments. This procurement relates to the peer review of a health risk assessment on the disinfection by product, chloroform. This risk assessment will be used in support of EPA's stage 1 disinfection by product rule which is scheduled to be final in November 1998. The Safe Drinking Water Act Amendments of 1996 emphasize that "the best peer review science" be used in carrying out SDWA regulations.

PURPOSE:

A cancer risk assessment/characterization document has been recently prepared that cites and updates EPA's 1994 assessment on chloroform. This 1998 document considers a new cancer bioassay in rodents and applies the EPA 1996 proposed revisions to its guidelines for carcinogen risk assessment.

TASK DESCRIPTION:

This purchase will procure a peer review on the 1998 EPA chloroform risk characterization assessment report. EPA has attached the 1998 risk assessment document, consisting of approximately 20-25 pages, on chloroform to be reviewed (Attachment 1), as well as supporting materials, such as EPA's 1996 guidelines for carcinogen assessment (Attachment 2), EPA's 1994 Criteria Document on chloroform (Attachment 3), and hard copies of key studies (Attachment 4). The peer reviewer shall submit written comments that are clear/transparent, and constructive. They shall comment on whether the document clearly and adequately discusses:

- the weight of the evidence
- the key lines of evidence
- the mode of carcinogenic action understanding
- alternative hypotheses
- uncertainties in the risk assessment

- scientific basis for the risk assessment dose-response choice (i.e., linear versus nonlinear default approaches)

The peer reviewers shall indicate where they are in agreement with the report and where they disagree. If they disagree with any part of the report or find a weakness in the report, they shall recommend explicit guidance on revising the report. They shall provide comments that include an overall general summary on the acceptability and adequacy of the risk assessment, and specific comments as needed (comment 1 on page X, paragraph X, line X).