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Ambient Water Quality Criterion for the Protection of Human Health: Chloroform - Revised Draft

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REVISED DRAFT

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Water Quality Criterion for the Protection of Human Health:

Chloroform

Revised Draft

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

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NOTE TO READER

The Agency is developing streamlined criteria documents which focus on critical toxicological and exposure-related studies only. This is a departure from the past format in which all existing toxicological and exposure studies were presented and evaluated, with equal emphasis placed on exposure, pharmacokinetics, toxicological effects, and criterion formulation. Due to limited resources and a need to update criteria as quickly as possible, EPA has decided to develop more abbreviated versions of criteria documents with an emphasis on using existing risk assessments (on IRIS or other EPA health assessment documents) where available and still relevant, and to focus to a greater extent on pertinent exposure and toxicological studies that may influence the development of a criterion (e.g., critical effects studies which form the basis of RfD development or cancer assessment). EPA will continue to conduct a comprehensive review of the literature for the latest studies, but will not provide a summary or an evaluation of the studies that are deemed less significant in the criteria development process. Where there is a significant amount of literature on an area of study (for instance, pharmacokinetics), EPA, to the extent possible, will reference the information or cite existing documents (e.g., IRIS or other existing EPA risk assessment documents) that discuss the information in greater detail.

The overall objective of this change in philosophy is to allow EPA to update 1980 ambient water quality criteria (AWQC) at a greater frequency, while still maintaining the scientific rigor that EPA requires when developing an AWQC. EPA believes these "new" criteria documents will be as informative as previous criteria documents and will continue to serve as the key scientific basis for State and Tribal standards. EPA also believes the documents will provide the necessary scientific content and scope to allow a State or Tribe to come to an appropriate technical and/or policy decision with regard to setting water quality standards.

EPA requests that commenters identify any relevant information missing from this criteria document which may result in a different criteria calculation or scientific interpretation.

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1. INTRODUCTION

Ambient Water Quality Criteria (AWQC) for chloroform were originally set in 1980 based on non-threshold carcinogenic effects (45 FR 79318). Because a non-threshold carcinogenic effect implies a risk of cancer at any concentration greater than zero, the ideal target concentration identified for chloroform in water was zero. However, because a level of zero may not be attainable, criteria were set based incremental increases in cancer risk. The criteria for an incremental risk of 10^{-6} were 0.19 µg/L for ingestion of water and organisms, and 15.7 µg/L for ingestion of organisms only.

Those criteria were revised in 1992 as part of the National Toxics Rule (57 FR 60848), based on a revised carcinogenic slope factor published in EPA's Integrated Risk Information System (IRIS) in 1988. The revised criteria for an incremental risk of 10^{-6} were 5.7 µg/L for ingestion of water and organisms, and 470 µg/L for ingestion of organisms only. These criteria are included in EPA's 2000 compilation of AWQC (USEPA 2000a).

As required under Section 304(a) of the Clean Water Act, EPA must periodically revise criteria for water quality to accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on human health from the presence of pollutants in any body of water. This criteria document updates the criteria for chloroform using new methods and new information described in the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA 2000b) and in the Methodology's accompanying *Federal Register Notice* (USEPA 2000c). These include new methods to determine toxicity dose-response relationships for both carcinogenic and noncarcinogenic effects, updated exposure factors (e.g., values for fish consumption), new exposure assumptions used in the calculation, and new procedures to determine bioaccumulation factors. The *Risk Assessment* Technical Support Document (TSD) accompanying the Federal Register notice describes the determination of toxicity dose-response relationships in greater detail (USEPA 2000d). In addition to the new methods and information described above, new information on toxicity, exposure, and bioaccumulation of chloroform is also included in this update.

Based on the most sensitive toxicity endpoint (cytolethality and regenerative hyperplasia in liver), the revised criterion is 68 µg/L to protect against ingestion of chloroform in drinking water and aquatic organisms, or 2,400 µg/L to protect against ingestion of chloroform in aquatic organisms alone. Based on available data on the mode of action, these criteria will protect exposed humans against both the noncancer and cancer effects of chloroform. The calculation is based on adults in the general population.

The following sections describe the toxicological, exposure, and bioaccumulation factor evaluations, and the calculation of the chloroform criteria.

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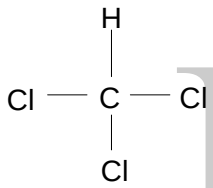
2. CHEMICAL AND PHYSICAL PROPERTIES

Revised AWQC are being derived for chloroform (CAS No. 67-66-3). The chemical formula is $CHCl_3$, and the chemical structure is:



Synonyms include trichloromethane and methane trichloride.

Physical and chemical properties (Howard and Meylan 1997, Montgomery and Welkom1989):

Chemical formula	$CHCl_3$
Molecular weight	119.38
Physical State (25°C)	Colorless, volatile liquid
Boiling Point	61.7°C
Density (20°C)	1.484 g/mL
Vapor pressure(25°C)	197 mm Hg
Specific Gravity (20°C/4°C)	1.483
Water solubility:	7.95 g/L at 25°C
Log Octanol:Water Partition Coefficient	1.97
Conversion factor (air concentrations)	1 ppm = 4.88 mg/m^3 1 mg/m^3 = 0.205 ppm

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3. SUMMARY OF TOXICOKINETICS

Data on the toxicokinetics of chloroform have been reviewed and summarized by USEPA (2001a). The text below is derived from USEPA (2001a) with little modification.

3.1 ABSORPTION

Studies in animals indicate that gastrointestinal absorption of chloroform is rapid (peak blood levels at about 1 hour) and extensive (64% to 98%) (USEPA 1997a, ILSI 1997, USEPA 1998c). Limited data indicate that gastrointestinal absorption of chloroform is also rapid and extensive in humans, with over 90% of an oral dose recovered in expired air (either as unchanged chloroform or carbon dioxide) within 8 hours (Fry et al. 1972).

Most studies of chloroform absorption following oral exposure have used oil-based vehicles and gavage dosing (USEPA 1994, 1998c). This is of potential significance because most humans are exposed to chloroform via ingestion in drinking water, and drinking water ingestion usually occurs intermittently throughout the day rather than as a single dose (bolus). Although the effect of administration of chloroform in oil is to slow absorption compared to gavage administration in water (Withey et al. 1983), the effect of gavage (bolus) dosing is to increase blood concentrations compared to intermittent exposures. Thus, extrapolation of dose-response data from non-drinking water studies should be done with caution because of potential pharmacokinetic differences.

Dermal and inhalation absorption of chloroform by humans during showering was investigated by Jo et al. (1990). Chloroform concentrations in exhaled breath were measured in six human subjects before and after a normal shower, and following inhalation-only shower exposure. Breath levels measured at 5 minutes following either exposure correlated with tap water levels of chloroform. Breath levels following inhalation exposure only were about half those following a normal shower (both inhalation and dermal contact). These data indicate that humans absorb chloroform by both the dermal and inhalation routes (USEPA 1994).

3.2 DISTRIBUTION

Absorbed chloroform appears to distribute widely throughout the body (USEPA 1994, 1998c). In postmortem samples from eight humans, the highest levels of chloroform were detected in the body fat (5–68 g/kg), with lower levels (1–10 g/kg) detected in the kidney, liver, and brain (McConnell et al. 1975). Studies in animals indicate rapid uptake of chloroform by the liver and kidney (USEPA 1997a).

3.3 METABOLISM

Chloroform is metabolized in humans and animals by cytochrome P450-dependent pathways. In the presence of oxygen (oxidative metabolism), the chief product is trichloromethanol, which rapidly and spontaneously dehydrochlorinates to form phosgene (CCl_2O):

$$2\ CHCl_3 + NADPH + H^+ + O_2 \rightarrow 2\ CCl_3OH + NADP^+$$
$$CCl_3OH \rightarrow CCl_2O + HCl$$

In the absence of oxygen (reductive metabolism), the chief metabolite is dichloromethyl free radical ($CHCl_2$•) (USEPA 1997a, ILSI 1997).

Nearly all tissues of the body are capable of metabolizing chloroform, but the rate of metabolism is greatest in liver, kidney cortex, and nasal mucosa (ILSI 1997). These tissues are also the principal sites of chloroform toxicity, indicating the importance of metabolism in the mode of action of chloroform toxicity.

At low chloroform concentrations, metabolism occurs primarily via cytochrome P450-2E1 (CYP2E1) (Constan et al. 1999). The level of this isozyme (and hence the rate of chloroform metabolism) is induced by a variety of alcohols (including ethanol) and ketones, and may be inhibited by phenobarbital. At high chloroform concentrations, metabolism is also catalyzed by cytochrome P450-2B1/2 (CYP2B1/2) (ILSI 1997; USEPA 1997a, 1998c). Because chloroform metabolism is enzyme-dependent, the rate of metabolism displays saturation kinetics. Under low dose-rate conditions, nearly all of a dose is metabolized. However, as the dose or the dose rate increases, metabolic capacity may become saturated and increasing fractions of the dose are excreted as the un-metabolized parent (Fry et al. 1972).

The products of oxidative metabolism (phosgene) and reductive metabolism (dichloromethyl free radical) are both highly reactive. Phosgene is electrophilic and undergoes attack by a variety of nucleophiles. The predominant reaction is hydrolysis by water, yielding carbon dioxide and hydrochloric acid. The rate of phosgene hydrolysis is very rapid, with a half-time of less than one second (De Bruyn et al. 1995). Phosgene also reacts with a wide variety of other nucleophiles, including primary and secondary amines, hydroxy groups, and thiols (Schneider and Diller 1991). For example, phosgene reacts with the thiol group of glutathione and with nucleophilic groups (-SH, -OH, $-NH_2$) in cellular macromolecules such as enzymes, proteins, or the polar heads of phospholipids, resulting in formation of covalent adducts (Pohl et al. 1977, 1980, 1981; Pereira and Chang 1981; Pereira et al.1984; Noort et al 2000).

Formation of these molecular adducts can interfere with molecular function (e.g., loss of enzyme activity), which in turn may lead to loss of cellular function and subsequent cell death (ILSI 1997, WHO 1998).

Free radicals that are formed under conditions of low oxygen are also extremely reactive, forming covalent adducts with microsomal enzymes and the fatty acid tails of phospholipids, probably quite close to the site of free radical formation (cytochrome P450 in microsomal membranes). This results in a general loss of microsomal enzyme activity, and can also result in lipid peroxidation (ILSI 1997, USEPA 1998c).

Two lines of evidence suggest that metabolism occurs mainly via the oxidative pathway. First, reductive metabolism of chloroform is observed only in phenobarbital-induced animals or in tissues prepared from them, with negligible reducing activity observed in uninduced animals (ILSI 1997). Second, in vitro studies using liver and kidney microsomes from mice indicate that, even under relatively low (2.6%) oxygen partial pressure (approximately average for the liver), more than 75% of the phospholipid binding was to the fatty acid heads. This pattern of adduct formation on phospholipids is consistent with phosgene, not free radicals, as the main reactive species, indicating metabolism was chiefly by the oxidative pathway (USEPA 1998c, ILSI 1997). Addition of glutathione to the incubation system completely negated binding to liver microsomes, with only residual binding remaining in kidney microsomes (ILSI 1997). This quenching by glutathione is expected for the products of oxidative but not reductive metabolism. Taken together, these observations strongly support the conclusion that chloroform metabolism in vivo occurs primarily via the oxidative pathway except under special conditions of high chloroform doses in pre-induced animals (ILSE 1997, USEPA 1998c).

3.4 EXCRETION

Excretion of chloroform occurs primarily via the lungs (USEPA 1998c). Results from studies in humans indicate that approximately 90% of an oral dose of chloroform was exhaled (either as chloroform or as carbon dioxide), with less than 0.01% of the dose excreted in the urine (USEPA 1994). In mice and rats, 45 to 88% of an oral dose of chloroform was excreted from the lungs either as chloroform or carbon dioxide, with 1–5% excreted in the urine (USEPA 1998c).

No data are available regarding the bioaccumulation or retention of chloroform following repeated exposure. However, due to the rapid excretion and metabolism of chloroform, combined with low levels of chloroform detected in human postmortem tissue samples, marked accumulation and retention of chloroform is not expected (USEPA 1994).

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4. TOXICOLOGICAL AND RISK BASES FOR CRITERIA

Data on the oral and inhalation toxicity of chloroform have been reviewed and summarized by USEPA (2001a). The text below is derived from USEPA (2001a) with little modification.

4.1 NONCANCER DATA AND PREVIOUS EVALUATIONS

The noncancer effects of chloroform have been investigated in a large number of studies in animals, with some supporting data from studies in humans. Oral exposure to chloroform is associated mainly with cytotoxicity to cells in the liver and kidney. Inhalation exposure is also associated with injury to liver and kidney, with neurological effects also occurring at high levels. Some of the studies supporting these conclusions are summarized below.

4.1.1 Oral Exposure

4.1.1.1 Oral Exposure Data in Humans

There have been several epidemiological studies that have investigated the association between human exposure to chloroform and other disinfection byproducts in chlorinated water and the occurrence of adverse reproductive outcomes (Kramer et al. 1992, Bove et al. 1995, Gallagher et al. 1998, Waller et al. 1998). Statistically significant correlations were observed between exposure to total trihalomethanes and one or more adverse reproductive outcomes, and in one case (Kramer et al. 1992), there was a significant relationship between chloroform levels and decreased intrauterine growth. In another case (Waller et al. 1998), an association was noted between increased risk of spontaneous abortion and bromodichloromethane (but not chloroform) levels. Although epidemiological studies of this type are useful in evaluating whether chlorinated drinking water can increase the risk of adverse reproductive effects in exposed populations, the studies are not adequate to establish a causal link between ingestion of chloroform and the occurrence of adverse reproductive effects in humans, because chlorinated drinking water contains many different potentially toxic disinfection byproducts.

4.1.1.2 Oral Exposure Data in Animals

Eschenbrenner and Miller (1945) exposed Strain A mice (5/sex/group) to chloroform at dose levels of 0, 150, 300, 600, 1200, or 2400 mg/kg-dat in olive oil by gavage. The animals were dosed every 4 days over a period of 120 days (a total of 30 doses). No

males administered doses of at least 600 mg/kg and no females in the high-dose group survived the study. All deaths occurred 24 to 48 hours after the first or second chloroform dose. Liver necrosis was observed in both sexes in the three highest dose groups. Males in all treatment groups developed kidney necrosis, whereas kidney necrosis was not apparent in any females. The severity of renal necrosis in males was dose related.

Palmer et al. (1979) exposed Sprague-Dawley rats (50/sex/group) to chloroform in toothpaste by gavage at doses of 0 or 60 mg/kg-day, 6 days/week for 80 weeks. No significant differences in mortality were observed between treated and control animals. A marginal decrease in body weight gain (about 10%) was observed in both treated males and females when compared with controls. A statistically significant decrease in relative liver weight was observed in treated females. Histologic examination of the liver revealed only minor changes, with no severe fatty infiltration, fibrosis, or marked bile duct abnormalities reported. The incidence of moderate to severe glomerulonephritis was reported to be slightly increased in treated males.

Heywood et al. (1979) exposed groups of eight male and eight female beagle dogs to chloroform at doses of 15 or 30 mg/kg-day. The chemical was given orally in a toothpaste base in gelatin capsules, 6 days/week for 7.5 years. This was followed by a 20- to 24-week recovery period. A group of 16 male and 16 female dogs received toothpaste base without chloroform and served as the vehicle control group. Eight dogs of each sex served as an untreated group and a final group of 16 dogs (8/sex) received an alternative non-chloroform toothpaste. Four male dogs (one each from the low- and high-dose chloroform groups, the vehicle control group, and the untreated control group) and seven female dogs (four from the vehicle control group and three from the untreated control group) died during the study. Although there was substantial variability in individual measurements, serum glutamate pyruvate transaminase (SGPT, now known as alanine aminotransferase or ALT) levels tended to be about 30%–50% higher in the low-dose group (15 mg/kg/day) than in control animals. These increases were statistically significant for weeks 130-364. For the high-dose group (30 mg/kg-day), the typical increase in SGPT was about twofold, and the differences were statistically significant for the entire exposure duration (weeks 6–372). After 14 weeks of recovery, SGPT levels remained significantly increased in the high-dose group but not in the low-dose group, when compared with the controls. After 19 weeks of recovery, SGPT levels were not significantly increased in either treated group when compared with the controls. The authors concluded that the increases in SGPT levels were likely the result of minimal liver damage. Serum alkaline phosphatase (SAP) and SGPT levels were also moderately increased (not statistically significant) in the treated dogs at the end of the treatment period when compared with the controls. Microscopic examinations were conducted on the major organs. The most prominent microscopic

effect observed in the liver was the presence of “fatty cysts,” which were described as aggregations of vacuolated histiocytes. The fatty cysts were observed in the control and treated dogs, but were larger and more numerous (i.e., higher incidence of cysts rated as “moderate or marked,” as opposed to “occasional or minimal”) in the treated dogs at both doses than in the control dogs. The prevalence of moderated or marked fatty cysts was 1/27 in control animals, 9/15 in low-dose animals, and 13/15 in high-dose animals. Nodules of altered hepatocytes were observed in both treated and control animals, and therefore were not considered related to treatment. No other treatment-related nonneoplastic or neoplastic lesions were reported for the liver, gall bladder, cardiovascular system, reproductive system, or urinary system. A NOAEL was not identified in this study. However, a LOAEL of 15 mg/kg/day was identified, based on elevated SGPT levels and increased incidence and severity of fatty cysts (USEPA 1998c).

Jorgenson and Rushbrook (1980) exposed seven groups of 6-week-old female B6C3F1 mice (30 mice/group) to drinking water containing either 0, 200, 400, 600, 900, 1800, or 2700 ppm chloroform for 30–90 days. Calculated dose levels were 0, 32, 64, 97, 145, 290, or 436 mg/kg-day based on reported water intakes. At week 1, a significant decrease in body weight was observed in the 900, 1800, and 2700 ppm chloroform treatment groups; however, all body weights of the treated animals were comparable to controls after week 1. On days 30, 60, and 90, ten animals from each treatment group were sacrificed for gross and microscopic pathologic examination, as well as for measurement of organ fat:organ weight ratios. A 160%–250% increase in liver fat was observed in the high-dose group. Histological examination of the liver revealed mild centrilobular fatty changes in the 1,800 and 2,700 ppm groups. On day 30, reversible fatty changes in the liver were observed at doses as low as 400 ppm chloroform. Treatment-related atrophy of the spleen was observed at the high dose. Based on the observation of mild effects of chloroform exposure via the drinking water on liver and other tissues, the LOAEL in this study was 290 mg/kg-day, while the NOAEL was 145 mg/kg-day (USEPA 1994).

Jorgenson et al. (1982) exposed male Osborne-Mendel rats and female B6C3F1 mice to chloroform in drinking water (0, 200, 400, 900, or 1,800 mg/L) for 1 to 6 months. The time-weighted average doses, based on measured water intake and body weights, were 0, 19, 38, 81, or 160 mg/kg-day in rats and 0, 34, 65, 130, or 263 mg/kg-day in mice. An additional group of matched controls received the same water volume as the high-dose groups. In male rats, some changes were observed in body weight and in some hematological and serum biochemical parameters, but the authors judged these changes to be a secondary effect of reduced water intake. Gross and microscopic pathology findings in the rats generally were slight or mild in severity, were not dose

related, and either appeared adaptive (occurred in rats sacrificed after 30 or 60 days, but not in those sacrificed after 90 days) or were sporadic (by nature and/or incidence) and not considered treatment-related. This study identifies a NOAEL of 160 mg/kg-day in the male rat. In mice, mortality within the first 3 weeks was significantly increased in the two highest dose groups (130 and 263 mg/kg-day), but was comparable to controls after that time. Early mortality and behavioral effects (e.g., lassitude, lack of vigor) were apparently related to reduced water consumption. A significant increase in liver fat in mice was noted at doses of 65 mg/kg-day and higher at 3 months, and at doses of 130 and 263 mg/kg-day at 6 months. This study identifies a NOAEL of 34 mg/kg-day and a LOAEL of 65-130 mg/kg-day in mice, based on increased liver fat at 3 to 6 months.

In the original study reports by Jorgenson et al. (1982, 1985), histological findings indicative of renal cytotoxicity were not included. Recently, histological slides of rat kidney from this study have been re-examined to assess whether evidence of renal cytotoxicity could be detected (ILSI 1997, Hard and Wolf 1999, Hard et al. 2000). Based on this reexamination, it was found that animals exposed to average doses of 81 or 160 mg/kg-day of chloroform displayed low-grade renal tubular injury with regeneration, mainly in the mid to deep cortex. The changes included faint basophilia, cytoplasmic vacuolation, and simple hyperplasia in proximal convoluted tubules. In some animals, single-cell necrosis, mitotic figures, and karyomegaly were also observed. Hyperplasia was visualized as an increased number of nuclei crowded together in tubule cross-sections. These changes were observable in the 160 mg/kg-day dose group at 12, 18, and 24 months, and in the 81 mg/kg-day dose group at 18 and 24 months. Cytotoxic changes were not seen in either of the lower dose groups (19 or 38 mg/kg-day). Based on histological evidence of renal cytotoxicity in rats, this study identifies a LOAEL of 81 mg/kg-day.

Bull et al. (1986) studied the effect of dose vehicle on the hepatotoxicity of chloroform using male and female B6C3F1 mice. Doses of 0, 60, 130, or 270 mg/kg-day in corn oil or in 2% emulphor were administered via gavage for 90 days. Based on measurements of serum enzyme levels, serum and tissue triglyceride levels, and histological examination of the livers, the authors concluded that hepatotoxic effects were enhanced by the administration of chloroform via corn oil versus chloroform administered in an aqueous suspension. The authors suggested that the cause may be absorption kinetics or interaction between chloroform and the corn oil vehicle (USEPA 1994). A LOAEL of 270 mg/kg-day was identified for chloroform when administered in corn oil, but 270 mg/kg-day was considered a NOAEL for chloroform when administered in aqueous vehicle (USEPA 1994).

Larson et al. (1994a) investigated the effects of oral exposure of female B6C3F1 mice to chloroform. Exposure occurred either through drinking water (0, 60, 200, 400, 900, or 1800 ppm) or by gavage in corn oil (0, 3, 10, 34, 90, 238, or 477 mg/kg-day) for up to three weeks. All animals were examined for histological lesions in the liver and for increases in hepatic labeling index (LI). In animals exposed by corn oil gavage, doses of 238 mg/kg-day or higher produced clear hepatic necrosis and increases in LI. No histological effects were observed at 10 mg/kg-day, and no increase in LI was seen at 34 mg/kg-day. In animals exposed to chloroform in water, there were no significant effects on LI nor were there any significant histological lesions at any exposure level, even though the effective dose was as high from drinking water as from gavage.

Larson et al. (1995) studied the effects of chloroform exposure on liver, kidney, and nasal passage in female F344 rats. Exposure occurred by gavage in corn oil for up to three weeks. Dose levels were 0, 34, 100, 200, or 400 mg/kg-day. Mild degenerative centrilobular changes and increases in LI were observed in the liver at doses of 100 mg/kg-day or higher. Degeneration and necrosis were observed in the kidney at doses of 200 mg/kg-day and higher, and increases in LI in kidney were observed at 100 mg/kg-day and higher. Effects on the peripheral region of the nasal turbinates (periostial hypercellularity and increased cell replication) were observed at all exposure doses.

4.1.1.3 Oral Reference Dose

EPA used the available data on the non-cancer effects of chloroform to estimate oral RfD values using two different approaches: the traditional NOAEL-LOAEL approach and the benchmark dose (BMD) modeling approach (USEPA 2001a). The results of these two evaluations are summarized below.

NOAEL-LOAEL Approach

For the NOAEL-LOAEL approach, the principal study selected to derive the RfD was the report by Heywood et al. (1979), in which there was an increase in the incidence of moderate to marked hepatic fatty cysts in dogs. This study was selected because it identifies the lowest LOAEL and because it is also the longest duration study (7.5 years). The lesions observed in this study were characterized by aggregations of vacuolated histiocytes. Although fatty cysts were observed in the control group as well as all treated groups, both the size and severity of these lesions were significantly increased in treated animals. The LOAEL for fatty cysts was 15 mg/kg-day, and a NOAEL was not identified. The LOAEL of 15 mg/kg-day was used to derive a chronic oral RfD for chloroform as follows:

$$RfD = \frac{15\ mg/kg\text{-}day \times (6\ days/7\ days)}{1{,}000} = 1E-02\ \ mg/kg\text{-}day$$

where:

15 mg/kg-day =	LOAEL identified by Heywood et al. (1979)
6 days/7 days =	Adjustment to account for exposure 6 days/week
1,000 =	Uncertainty factor. This uncertainty factor includes a factor of 10 to extrapolate from a LOAEL to a NOAEL, a factor of 10 to extrapolate from an animal species (dog) to humans, and a factor of 10 to account for potential sensitive human subpopulations.

Benchmark Dose Approach

In accord with EPA guidance (USEPA 1995), several data sets in addition to the data set with the lowest LOAEL (Heywood et al. 1979) were selected for benchmark dose (BMD) modeling. This is because the study that identifies the lowest LOAEL may not always be suitable for modeling or might not always yield the lowest BMD. The data sets selected for modeling included (a) incidence of fatty cysts in liver of dogs (Heywood et al. 1979), (b) histological evidence of renal cytotoxicity in male rats exposed via drinking water (Hard et al. 2000), (c) increased labeling index in kidney of female mice exposed via drinking water (Larson et al. 1994a), and (d) increased labeling index in liver of female rats exposed via gavage in corn oil (Larson et al. 1995).

Based on the BMD fitting, a BMDL value of 1.2 mg/kg-day derived from the study by Heywood et al. (1979) was selected as the most appropriate basis for the derivation of the RfD. Because this value is based on exposures that occurred 6 days/ week, the value was adjusted as follows:

$$BMDL = (1.2\ mg/kg\text{-}day) \times (6/7) = 1.0\ mg/kg\text{-}day.$$

The RfD was derived from the BMDL by application of appropriate uncertainty factors. In this case, an uncertainty factor of 10 is used to account for interspecies extrapolation and a factor of 10 is used to protect potentially sensitive human subpopulations. Additional uncertainty factors are not required because the database for chloroform is complete. Bioassays are available in the dog (Heywood et al. 1979) and the rat and mouse (NCI 1976; Jorgenson et al. 1982, 1985). Developmental toxicity studies are available in rats and rabbits exposed via the oral route (Thompson et al. 1974), and in rats (Baeder and Hoffmann 1988, 1991; Schwetz et al. 1974; Stanford Research

Institute 1978) and mice (Murray et al. 1979) exposed by the inhalation route. These studies indicate that effects on the fetus do not occur except at doses that cause maternal toxicity. A two-generation reproduction study (NTP 1988) found no effects on fertility or reproduction at doses that resulted in liver histopathology. Finally, chloroform is rapidly metabolized and excreted and thus is not expected to bioaccumulate. Based on all of these considerations, a total uncertainty factor of 100 was applied and the resulting RfD was 1E-02 mg/kg-day:

RfD = 1.0 *mg/kg-day* / 100 = 1E-02 *mg/kg-day.*

In general, the choice between the NOAEL-LOAEL approach and the BMD approach depends on the quality of data and confidence in the fitting results. However, in this particular case, the two approaches (NOAEL/LOAEL, benchmark) yield equal RfD values.

4.1.2 Inhalation Exposure

4.1.2.1 Use of Chloroform as an Anesthetic

Chloroform was used from around 1850 to 1950 as an anesthetic in midwifery and surgery. It was generally preferred to ether because of its ease of use and its non-flammable nature. However, in the initial period of use, a number of deaths occurred, which were mainly attributed to cessation of the heart following over-exposure to chloroform vapor (Davidson 1965). Once the potential for harm was recognized, physicians and scientists at the time recommended that use of chloroform as an anesthetic be restricted to airborne concentrations in the 2%-4% range (20,000-40,000 ppm). Whitaker and Jones (1965) reported on the outcome of over 1,500 patients who had undergone chloroform anesthesia in more recent years, with the highest concentration being 2.25% (22,500 ppm). Effects observed following administration of chloroform included increased respiratory rate (44%), bradycardia (the incidence increasing from 7% at less than 30 minutes exposure to 25% at more than 120 minutes exposure), cardiac syncope (1%), hypotension (33%), nausea and vomiting (7%), jaundice (1 patient, probably from hepatitis), and death (one patient, probably from infection). This body of clinical experience demonstrates that central nervous system depression is the chief effect of acute inhalation exposure to high concentrations of chloroform, and that clinically significant effects on target tissues such as liver are unlikely to be observed following brief exposures (generally less than a few hours).

4.1.2.2 Inhalation Studies in the Workplace

Several epidemiological studies have been performed to investigate the occurrence of adverse effects in populations of workers exposed to chloroform vapors in the workplace (Bomski et al. 1967, Challen et al. 1958, Phoon et al. 1983, Li et al. 1993). All of these studies are limited by lack of detailed exposure information for the study subjects, and hence the available data are not adequate to define with confidence the inhalation dose-response curve for either neurological or hepatic effects in humans. However, the data indicate that inhalation exposure of workers to chloroform can produce a range of neurological effects including fatigue, nausea, vomiting, lassitude, dry mouth, and anorexia. Effects on the liver (jaundice, increased serum enzyme levels, hepatomegaly) were also observed in some studies (Phoon et al. 1983, Bomski et al. 1967).

4.1.2.3 Inhalation Studies in Animals

Mery et al. (1994) exposed rats and mice to chloroform for 6 hours/day for 7 consecutive days. Target exposure concentrations ranged from 1 to 300 ppm. Examination of the nasal passages revealed that chloroform caused a complex set of responses in the ethmoid turbinates, predominantly in rats. These lesions were most severe peripherally and generally spared the tissue adjacent to the medial airways. The changes were characterized by atrophy of Bowman's glands, new bone formation, and increased labeling index in periosteal cells. The only change noted in the mouse was increased cell proliferation without osseous hyperplasia. The NOAEL values for these responses ranged from 3 to 100 ppm, with histological and induced cell proliferation being the most sensitive indices of effect.

Larson et al. (1996) exposed male and female B6C3F1 mice to chloroform in air at target concentrations of 0, 0.3, 2, 10, 30, or 90 ppm. Exposure was for 6 hours/day, 7 days/week, for 4 days or for 3, 6, or 13 weeks. Some additional animals were exposed 5 days/week for 13 weeks. All animals were examined for histological lesions of liver, kidney, and nasal epithelium. Some animals were administered bromodeoxyuridine (BrdU) via osmotic pump prior to sacrifice in order to measure the labeling index (LI). Chloroform caused treatment-related histopathological lesions in liver and nasal passages of male and female mice and the kidneys of male mice. Lesions in liver were characterized by centrilobular hepatocyte swelling and lipid vacuolization along with scattered enlarged nuclei and individual cell necrosis. The NOAEL for histological effects in liver was 10 ppm, and the LOAEL was 30-90 ppm. Renal lesions occurred in male but not female mice, primarily in the epithelial cells of the proximal convoluted tubules in the cortex. Changes included mineralization, enlarged nuclei, and scattered areas of regenerating foci. The NOAEL for histological effects in kidney was 10 ppm,

and the LOAEL was 30-90 ppm. Nasal effects occurred in animals exposed for 4 days at 10 ppm or higher. Nasal lesions were characterized by mild proliferative responses of epithelial cells along with a thickening of bone. Increases in LI were also observed at 10 ppm and higher. Both the histological and LI effects on nasal tissue were transient, with little or no difference from controls after 13 weeks of exposure.

Templin et al. (1996a) exposed male and female F344 rats to chloroform in air at target concentrations of 0, 2, 10, 30, 90, or 300 ppm. Exposure occurred for 6 hours/day, either 5 or 7 days/week, for 4 days or 3, 6, or 13 weeks. Additional animals were exposed for 5 days/week for 13 weeks. All animals were examined for histological lesions of liver, kidney, and nasal epithelium. Some animals were administered bromodeoxyuridine (BrdU) via osmotic pump prior to sacrifice in order to measure the LI. Exposure to chloroform caused histopathological lesions in liver, kidney, and nasal epithelium of both male and female rats. Lesions in liver were characterized by scattered individual hepatocyte degeneration and necrosis, mitotic figures, and midzonal vacuolization. The NOAEL for histological effects in liver was typically 30 ppm, and the LOAEL was typically 90 ppm. Renal lesions occurred primarily in the epithelial cells of the proximal convoluted tubules in the cortex. Changes included scattered vacuolation, individual tubule cell necrosis, and enlarged epithelial cell nuclei. The NOAEL for histological effects in kidney was 30 to 90 ppm, and the LOAEL was 90 to 300 ppm. Increases in renal LI occurred at 30 ppm when exposure was 7 days/week, and at 90 ppm when exposure was 5 days/week. Nasal lesions were characterized by atrophy of olfactory epithelium, mainly in the ethmoid portion of the nasal passage. Effects were minimal at 2 ppm and increased in severity at higher exposure levels. Increases in LI were seen in nasal epithelium at 10 ppm but not at 2 ppm. These effects tended to be much larger after 4 days of exposure than after 3-13 weeks of exposure.

Templin et al. (1998) exposed male BDF_1 mice to chloroform in air at target concentrations of 0, 1, 5, 30 or 90 ppm. Female BDF_1 mice were exposed to 0, 5, 30 or 90 ppm. Animals were exposed for 6 hours/day, 5 days/week, for 3-13 weeks. All animals were examined for histological lesions of liver and kidney. Effects on nasal epithelium were not investigated. Some animals were administered bromodeoxyuridine (BrdU) via osmotic pump prior to sacrifice in order to measure the LI. Exposure to chloroform caused histopathological lesions in liver of male and female mice. Liver lesions were characterized by centrilobular swelling along with centrilobular and midzonal vacuolization and degeneration. The NOAEL for histological effects in liver was 5 ppm, and the LOAEL was 30 ppm. In males, increases in hepatic LI were observed at 90 ppm at 7 weeks but not at 13 weeks. In females, an increase in LI was observed at 90 ppm at 13 weeks. No effects on hepatic LI were seen at 30 ppm at any time. Renal lesions were observed in male but not female mice. The predominant

alteration was replacement of some or most of the epithelial cells of the proximal convoluted tubules with regenerating cells characterized by basophilic cytoplasm and variably sized heterochromatic nuclei. The NOAEL for histological effects in kidney was 5 ppm, and the LOAEL was 30 ppm. Increases in renal LI were observed at 30 ppm but not 5 ppm.

Constan et al. (2002) exposed groups of female B6C3F1 mice (5 per group) to chloroform vapors of 0, 10, 30, or 90 ppm for 2, 6, 12, or 18 hours/day for 7 consecutive days. Animals were observed for clinical signs during exposure and were subjected to gross necropsy and histopathological examination at sacrifice. All animals were administered bromodeoxyuridine (BrdU) via osmotic pump prior to sacrifice in order to measure LI. Groups of mice exposed to 90 ppm for 6 hours/day or to 30 ppm for 6 or 12 hours/day had minimal to mild hepatopathology characterized by centrilobular and midzonal hepatocytic vacuolar degeneration. Individual necrotic cells were also observable. Statistically significant increases in LI were observed in these three groups, as well as animals exposed to 90 ppm for 2 hours/day. No histological effects or increases in LI occurred in any group exposed to 10 ppm, regardless of exposure duration. The authors concluded that at an exposure level of 10 ppm, the rate of production of toxic metabolites does not exceed the rate at which cellular detoxification and repair reactions can occur. Based on physiologically-based pharmacokinetic dose modeling, the authors estimated that the no-effect concentration for liver effects in humans would be about 110 ppm.

4.1.2.4 Inhalation Reference Concentration

The EPA is currently working to develop an inhalation RfC for chloroform, but a consensus value has not yet been established.

4.2 CANCER EVALUATION

4.2.1 Oral Exposure

4.2.1.1 Studies in Humans

At present, there have been no studies of cancer incidence in humans chronically exposed to chloroform (alone). However, there have been a number of epidemiological studies on cancer risk in humans who are exposed to chlorinated drinking water (e.g., Cantor et al. 1985, McGeehin et al. 1993, King and Marrett 1996, Doyle et al. 1997, Freedman et al. 1997, Cantor et al. 1998, Hildesheim et al. 1998). Chlorinated drinking water typically contains chloroform, along with other trihalomethanes and a wide variety of other disinfection byproducts (USEPA 1994). It should be noted that humans

exposed to chloroform in drinking water are likely to be exposed both by direct ingestion and by inhalation of chloroform gas released from water into indoor air.

Some of these epidemiological studies have detected a weak association between exposure to chlorinated water and cancer (mainly bladder cancer). Based on the studies of Cantor et al. (1985), McGeehin et al. (1993), King and Marrett (1996), Freedman et al. (1997) and Cantor et al. (1998), EPA calculated that the population attributable risk (the fraction of a disease which could be eliminated if the exposure of concern were eliminated) for bladder cancer ranged from 2% to 17% (USEPA 1998f). However, these calculations are based on a number of assumptions, including the assumption that there is a cause-effect relationship between exposure to chlorinated drinking water and increased risk of bladder cancer. This assumption is subject to considerable uncertainty, especially since findings are not consistent within or between studies. Evaluation of these studies by application of standard criteria for establishing causality from epidemiological observations (strength of association, consistency of findings, specificity of association, temporal sequence, dose-response relation, biological plausibility) has led EPA to conclude that the current data are insufficient to establish a causal relationship between exposure to chlorinated drinking water and increased risk of cancer (USEPA 1998f). Moreover, even if, in the future, the weight of evidence does become sufficient to establish a causal link between exposure to chlorinated water and increased risk of bladder or other types of cancer, it could not be concluded from epidemiological studies of this type that chloroform per se is carcinogenic in humans, since chlorinated water contains numerous disinfection byproducts besides chloroform that are potentially carcinogenic (USEPA 1994).

4.2.1.2 Studies in Animals

There have been a number of studies in animals that demonstrate that ingestion of chloroform may increase the risk of cancer, at least by some types of exposure. The most important of these studies are summarized below.

NCI (1976) evaluated the carcinogenic potential of chloroform in Osborne-Mendel rats. Male rats were administered concentrations of 90 or 180 mg chloroform/kg-day in corn oil, via oral gavage, 5 days/week for 78 weeks. Female rats were administered concentrations of 125 or 250 mg/kg-day for 22 weeks, after which the doses were reduced to 90 or 180 mg/kg-day, with the average dose over the course of the study being 100 or 200 mg/kg-day. Three additional groups of animals served as matched, colony, and positive controls. At week 111, all rats were sacrificed. A statistically significant increase in the incidence of kidney epithelial tumors was observed in male rats in the high-dose group (12/50) when compared with males in the control group (0/98). A statistically significant increase in the incidence of thyroid tumors was also

observed in female rats, but this finding was not considered biologically significant (USEPA 1994).

NCI (1976) also evaluated the carcinogenic potential of chloroform using male and female B6C3F1 mice. The average dose levels for the study were 138 or 277 mg/kg-day for males and 238 or 477 mg/kg-day for females. All mice were sacrificed at weeks 92 or 93. Three additional groups of animals served as matched (20/sex/group), colony (99 males and 98 females), and positive (100/sex/group) controls. The incidence of hepatocellular carcinomas was significantly increased in males in both the low-dose (18/50) and high-dose (44/45) groups when compared to controls (5/77). A similar increase was observed for low-dose (36/46) and high-dose (39/41) females compared to control (1/80) Many of the male mice in the low-dose group that did not develop hepatocellular carcinoma had nodular hyperplasia of the liver. The incidence of kidney epithelial tumors was comparable between treatment and control groups.

Roe et al. (1979) reported three experiments in mice to evaluate the potential carcinogenicity of chloroform. In three different studies, 10-week-old mice were administered chloroform by gavage in a toothpaste base 6 days/week for 80 weeks, followed by a 13- to 24-week observation period. The design of each study is summarized below:

Study	Strain (gender)	N	Doses (mg/kg-day)
I	ICI (male, female)	52/sex	0, 17, 60
II	ICI (male)	52	0, 60
III	C57BL, CBA, CF/1, ICI (male)	52 per strain	0, 60

In experiment I, kidney tumors were statistically higher in high-dose male mice than in controls, while all other tumor incidences were comparable to the controls. In experiment II, a decrease in liver and kidney weights was observed in chloroform-treated male mice, and the incidence of kidney tumors was increased. In experiment III, treatment with chloroform was associated with increased incidence of moderate to severe kidney lesions in CBA and CF/1 mice. No increases in liver or kidney tumors were observed except in ICI male mice.

Heywood et al. (1979) exposed groups of 8 male and 8 female beagle dogs to doses of 15 or 30 mg chloroform/kg-day. The chemical was given orally in a toothpaste base in gelatin capsules, 6 days/week for 7.5 years. This was followed by a 20- to 24-week recovery period. A group of 16 male and 16 female dogs received toothpaste base without chloroform and served as the vehicle control group. Eight dogs of each sex

served as an untreated group and a final group of 16 dogs (8/sex) received an alternative nonchloroform toothpaste. No treatment-related neoplastic lesions were reported for the liver, gall bladder, cardiovascular system, reproductive system, or urinary system.

Jorgenson et al. (1985) exposed male Osborne-Mendel rats and female B6C3F1 mice to chloroform in drinking water (0, 200, 400, 900, or 1,800 mg/L) for 104 weeks. Time-weighted average doses, based on measured water intake and body weights, were 0, 19, 38, 81, or 160 mg/kg-day for rats and 0, 34, 65, 130, or 263 mg/kg-day for mice. An additional group of animals that served as controls was limited to the same water intake as the high-dose groups. The number of animals in the dose groups (from low to high) was 330, 150, 50, and 50 for rats and 430, 150, 50, and 50 for mice. A statistically significant dose-related increase in the incidence of kidney tumors (tubular cell adenomas and adenocarcinomas) was observed in male rats in the high-dose group (160 mg/kg-day). Chloroform in the drinking water did not increase the incidence of hepatocellular carcinomas in female B6C3F1 mice. The combined incidence of hepato-cellular adenomas and carcinomas was 2% in the high-dose group compared with 6% in the control groups. The authors speculated that the differences observed between this study and the NCI (1976) bioassay may be related to differences in the mode of administration (in drinking water versus in corn oil by gavage).

Tumasonis et al. (1987) exposed male and female Wistar rats to chloroform in drinking water at concentrations of 0 or 2,900 mg/L for 72 weeks. Concentrations of chloroform were then reduced to 1,450 mg/L for an additional 113 weeks until all animals had died (approximately 185 weeks). The average dose for males and females was approximately 200 and 150 mg/kg-day, respectively (USEPA 1994). Treated females (but not males) showed a statistically significant increase in the incidence of hepatic neoplastic nodules, and both males and females had a statistically significant increase in the incidence of hepatic adenofibrosis. It is unclear if the nodules and adenofibroses were considered to be tumors (USEPA 1994).

DeAngelo (1995) exposed male F-344 rats to chloroform in drinking water for 100 weeks. Exposure levels were 0, 900, or 1,800 ppm. Assuming ingestion of about 0.05 L/day of water per kg body weight, this corresponds to doses of approximately 45 and 90 mg/kg-day, respectively. Exposure began when the animals were 8 to 10 weeks of age. Interim sacrifices of groups of 6 animals were performed at 26, 52, and 78 weeks, and the final sacrifice at 100 weeks included 50 animals per group. At each time point, liver and kidney were examined for gross and microscopic lesions. In the liver, there were borderline significant (p = 0.05 to 0.10) increases in the prevalence of hepatocellular proliferative lesions at 100 weeks. In addition, there was a statistically significant increase ($p < 0.05$) in the multiplicity of hepatic adenomas and carcinomas in

the group exposed to 1,800 ppm, and a significant dose trend ($p < 0.05$) for hyperplastic nodules, neoplasia, and total proliferative lesions. No renal neoplasms were observed in any of the chloroform-exposed groups.

4.2.2 Inhalation Exposure

4.2.2.1 Studies in Humans

No studies were located in humans on inhalation exposure to chloroform and increased risk of cancer.

4.2.2.2 Studies in Animals

Nagano et al. (1998) evaluated the effects of chronic exposure of F344 rats and BDF1 mice to chloroform vapor. This study has also been summarized in abstract form by Yamamoto et al. (1994, and described in a letter report by Matsushima (1994). Groups of male and female rats and mice were exposed to target chloroform vapor concentrations of 0, 10, 30, or 90 ppm (rats) or 0, 5, 30, or 90 ppm (mice), 6 hours/day, 5 days/week for 104 weeks. To avoid lethality in the high-dose groups, mice in the 30-ppm group were exposed to chloroform concentrations of 5 ppm for the first 2 weeks, 10 ppm for the next 2 weeks, and then exposed to 30 ppm for the remaining period. For the 90 ppm group, rats were exposed to chloroform initially at 10 ppm for 2 weeks, then 30 ppm for 2 weeks, and then 90 ppm for 98 weeks. The time-weighted average for the 30-ppm group was 29.1 ppm and for the 90-ppm group 85.7 ppm. Statistically significant increases in the incidence of renal cell adenoma and renal cell carcinoma (combined) were observed in male mice in the 30 (7/50) and 90 (12/48) ppm groups when compared with controls (0/50). The overall incidence rates of renal cell carcinoma were statistically significantly increased in males in the 90-ppm group (11/48) when compared with controls (0/50). There were no statistically significant changes in tumor incidence for female mice or for male or female rats in any exposure group.

4.2.3 Mutagenicity

A large number of studies have been performed to evaluate the mutagenicity of chloroform. These data have been reviewed and evaluated by several groups of experts, including the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC), ILSI (1997), and WHO (1998).

ICPEMC applied a comprehensive, quantitative weight-of-evidence approach to characterize the genotoxic potential of more than 100 chemicals with large genetic toxicity databases (Lohman et al. 1992). In this approach, scores are developed for

relative DNA reactivity. For a particular chemical, the maximum possible score is 100 and the minimum possible score is –100. The highest actual score obtained using this approach was 49.7 (triazaquone) and the lowest score was –27.7 (ethanol). When this approach was applied to chloroform, the score based on the results of more than 40 studies was –14.33. Thus, ICPEMC concluded that the weight of evidence indicates that chloroform should be classified as nongenotoxic (Brusick et al. 1992, Lohman et al. 1992).

ILSI (1997) performed a review of the available data on the mutagenicity of chloroform. The committee noted that phosgene is highly reactive and might be expected to have the capacity to interact directly with DNA, but that phosgene has not been tested in any standard mutagenicity test system. The committee also noted that, because of its high reactivity, phosgene formed in the cytosol following chloroform metabolism would likely react with cellular components prior to reaching the cell nucleus, and concluded that direct effects on DNA would be unlikely. Based on their review of the available data, the ILSI committee concluded that no subset of observations points unequivocally to a specific genotoxic mode of action associated with chloroform, and that the preponderance of the evidence indicates that chloroform is not strongly mutagenic (ILSI 1997). Based on this, the committee concluded that chloroform would not be expected to produce rodent tumors via a genotoxic mechanism.

WHO (1998) noted that studies on the mutagenicity of chloroform must be considered in light of the fact that (1) chloroform is volatile, so tests that do not prevent volatilization are unreliable, and (2) most chloroform contains ethanol, which may react with phosgene generated from chloroform metabolism to yield ethyl or diethyl carbamates (potentially causing false positive results). The WHO committee noted that largely negative results have been obtained in *Salmonella typhimurium* and *Escherichia coli* (with and without activation), in gene mutation tests in Chinese hamster ovary (CHO) cells and human lymphocytes, in mouse micronucleus tests, and in tests of unscheduled DNA synthesis both *in vitro* and *in vivo*. Given the large number of sensitive assays that have been used to investigate the genotoxicity of chloroform, the committee considered it noteworthy that the positive responses were so few, and that the positive results were randomly distributed among the various assays. Taken together, WHO (1998) concluded that the weight of evidence indicates that neither chloroform nor its metabolites appear to interact directly with DNA or possess genotoxic activity.

EPA considered all of the available data and noted that the results of the mutagenicity assays that have been conducted with chloroform are mixed (USEPA 2001a). By number, the majority of tests are negative, and many of the positive studies have been

conducted under high exposure conditions that resulted in severe cytotoxicity. As expressed by EPA's Science Advisory Board (SAB 2000):

> *Genotoxicity endpoints have to be interpreted cautiously when used as evidence for potential carcinogenicity. In vitro clastogenicity can be a product of severe cytotoxicity resulting from lysosomal or other releases (Brusick 1986). This may be important with substances such as chloroform, where there is evidence of cytotoxicity and cell proliferation in target tissues. Also, cycles of cytotoxicity and cell proliferation could cause the expression of preexisting genetic damage in target tissues which, under normal conditions, have low mitotic indices.*

Consequently, EPA concluded that the relevance of many of the positive studies is questionable. Therefore, based on the preponderance of negative findings and the uncertain relevance of the positive findings, EPA concluded that the weight of evidence indicates that even though a role for mutagenicity cannot be excluded with certainty, chloroform is not a strong mutagen and that neither chloroform nor its metabolites readily bind to DNA (USEPA 2001a). Based on these results and the results of studies that evaluated other endpoints of DNA reactivity, it seems likely that chloroform does not produce carcinogenic effects primarily by a specific mutagenic mode of action.

4.2.4 Cancer Evaluation Using Current Guidelines

In accord with proposed EPA guidelines for cancer risk assessment (USEPA 1996a), the method used to characterize and quantify cancer risk from a chemical depends on what is known about the mode of action of carcinogenicity and the shape of the cancer dose-response curve for that chemical. A default assumption of linearity is appropriate when evidence supports a mode of action of gene mutation due to DNA reactivity or supports another mode of action that is anticipated to be linear. The linear approach is used as a matter of policy if the mode of action of carcinogenicity is not understood. A default assumption of non-linearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of nonlinearity. Alternatively, the mode of action may theoretically have a threshold, e.g., the carcinogenicity may be a secondary effect of toxicity that is itself a threshold phenomenon (USEPA 1996a).

USEPA (2001a) has completed an evaluation of the carcinogenic hazard of chloroform, using EPA's new cancer assessment guidelines. The following sections summarize this evaluation, taken from USEPA (2001a) with little modification.

4.2.4.1 Mode of Action

Noncancer Mode of Action

The exact mode of action by which chloroform produces toxic effects in liver and kidney is not yet certain, but it is evident that metabolism of chloroform to toxic metabolites by cytochrome P450-dependent pathways plays a critical role (USEPA 1994, USEPA 2001a). For example, Brown et al. (1974) reported that pretreatment of rats with phenobarbital (a cytochrome P-450 inducer) resulted in increased hepatic toxicity following chloroform exposure. Similarly, Gopinath and Ford (1975) indicated that chloroform hepatotoxicity in rats was increased by phenobarbitone, phenylbutazone, and chlorpromazine, all inducers of microsomal enzymes. Conversely, inhibitors of microsomal enzymes, such as SKF-525A, sodium diethyl-dithiocarbamate, and carbon disulfide, decreased the hepatic toxicity of chloroform. Constan et al. (1999) showed that 1-aminobenztriazole, which is a general cytochrome P450 inhibitor, prevented chloroform-induced toxicity in liver and kidney of mice following inhalation exposure.

Further evidence of the role of metabolism is derived from the finding that variations in toxicity between tissues, genders, and species generally correlate with differences in cytochrome P450 metabolic rate. For example, male mice are more sensitive to chloroform-induced renal toxicity than female mice, and this difference in toxicity is paralleled in a difference in metabolism in proximal tubular cells (Ilett et al. 1973). Renal cytochrome levels in mice are increased by testosterone (Mohla et al. 1988, Henderson et al. 1989, Hong et al. 1989), and female mice treated with testosterone have increased renal toxicity along with increased covalent binding of chloroform metabolites (Taylor et al. 1974, Smith et al. 1979, Pohl et al. 1984). Conversely, male mice that were castrated had lower levels of chloroform-derived radioactivity accumulated in the kidneys (Eschenbrenner and Miller 1945, Culliford and Hewitt 1957, Taylor et al. 1974, Smith et al., 1984). Constan et al. (1999) compared the toxicity of chloroform in three strains of mice: B6C3F1, Sv/129 wild type, and Sv/129 CYP2E1 knockout mice. Exposure to 90 ppm chloroform for 6 hours/day for 4 days produced clear hepatotoxicity and renal toxicity (histopathology, increased labeling index) in the B6C3F1 mice and the Sv/129 wild type, but not in the Sv/129 CYP2E1 knockout mice. The authors concluded that metabolism of chloroform by CYP2E1 was obligatory for toxicity, at least at the dose tested.

Cancer Mode of Action

As noted above, studies in animals reveal that chloroform can cause an increased incidence of kidney tumors in male rats and an increased incidence of liver tumors in male and female mice. However, not all exposure regimens have been observed to

produce increases in cancer incidence. In particular, increase in tumors appear to occur only in those cases where the exposure regimen resulted in cytotoxicity, and did not occur under conditions when cytotoxcity did not occur (Butterworth and Bogdanffy 1999). This observation lead to the hypothesis that chloroform-induced tumor responses are secondary to cytotoxicity, most likely as a consequence of the regenerative hyperplasia which occurs following cytotoxicity in liver and kidney.

Regenerative hyperplasia can lead to an increased probability of cancer by one or both of two alternative modes of action. First, cells that are undergoing cell division are inherently more susceptible to initiation than are slowly growing or nondividing cells. This is because DNA undergoing replication is more exposed to nucleophilic attack than DNA that is covered with histones and arranged in nucleosomes (Ames and Gold, 1991a,b). Also, any gene damage that occurs in a cell undergoing division has less time to be repaired before mitosis than in a slowly growing cell, so a larger fraction of DNA alterations could be converted into mutations. Second, chemicals that promote cell division may convey a selective growth advantage to preexisting initiated cells in comparison with normal cells, thereby facilitating clonal expansion of initiated cells. This could occur because initiated cells are more responsive than normal cells to growth stimuli, because they are less susceptible to the toxicity of the chemical, or because they are less susceptible to endogenous regulatory signals that trigger programmed cell death (apoptosis). In any case, the ratio of cell birth to cell death of initiated cells increases compared with normal cells, leading to increased likelihood that a clone of initiated cells will form and survive. A key characteristic of this mode of action is that the effect is reversible: the clones of induced cells will tend to regress if the promoter (mitogen, cytotoxicant) is withdrawn (Pitot et al. 1987, Schulte-Hermann et al. 1993).

EPA reviewed the strength and consistency of the association between cytotoxicity and regenerative hyperplasia and the occurrence of increased tumor frequency in exposed animals (USEPA 2001a). This analysis revealed two main points:

- There are numerous cases where exposure to chloroform causes an increase in cellular regeneration (as reflected in an increase in LI) without any observable increase in cancer incidence. These data indicate that chloroform exposures that are adequate to cause cytotoxicity and regenerative cell proliferation do not always lead to cancer.

- There are no cases in which a tumorigenic response has been observed where evidence of cell regeneration is not also observed at the same or lower dose as that which caused an increase in tumors. This consistency of evidence (i.e., cell regeneration is detected in all cases of tumorigenicity) is strong evidence

supporting the conclusion that cell regeneration is a mandatory precursor for tumorigenicity.

In male Osborne-Mendel rats exposed to chloroform in water for 2 years (Jorgenson et al. 1985), a statistically significant increase in renal tumors was observed at a concentration of 1,800 ppm (160 mg/kg-day). A reanalysis of the histopathological slides from this study (Hard et al. 2000) revealed evidence for sustained cytotoxicity and cell proliferation in the kidney at exposures of 900 ppm (81 mg/kg-day) or higher. Likewise, in BDF1 mice exposed to chloroform by inhalation at 5, 30, or 90 ppm for 6 hours/day, 5 days/week (Nagano et al. 1998), increased incidence of renal tumors was observed in male mice at the two higher doses while females showed no significant tumor response. Templin et al. (1998) duplicated this exposure regimen to study whether the treatment caused cytotoxicity and regenerative hyperplasia. These authors observed cytotoxicity and hyperplasia in the kidneys of male mice exposed to 30 or 90 ppm throughout a 90-day exposure period, but not in exposed females. This observation is consistent with the hypothesis that sustained cytotoxicity and regenerative hyperplasia are key events in the neoplastic response of the kidney to chloroform.

Available data also indicate that cytotoxicity and regenerative hyperplasia are required for liver cancer, although the strength of this conclusion is somewhat limited because most of the observations are based on short-term rather than long-term histological or LI measurements. For example, in B6C3F1 mice, exposure by corn oil gavage at the same doses that resulted in liver tumors in the study by NCI (1976) also caused hepatic cytolethality and a cell proliferative response at 4 days and 3 weeks (Larson et al. 1994a,b). Similarly, exposure of female B6C3F1 mice to chloroform in drinking water at levels that did not induce liver tumors (Jorgenson et al. 1985) also did not induce hepatic cytolethality or cell proliferation at 4 days or 3 weeks (Larson et al. 1994a). This consistency of the data (i.e., evidence of cytolethality and/or regenerative hyperplasia is always observed in cases of increased liver tumors) supports the conclusion that this liver cancer also occurs via a mode of action involving regenerative hyperplasia.

In summary, based on a review of all available data, EPA concluded that the weight of the evidence in animals supports the conclusion that chloroform-induced tumors in liver and kidney are only produced at dose levels that result in repeated or sustained cytotoxicity and regenerative cell proliferation, and that direct genotoxicity is unlikely to play an important role (USEPA 2001a). No data exist that indicate the mode of action observed in rodents does not also apply to humans.

4.2.4.2 Quantification of Cancer Risk from Oral Exposure

The *Proposed Guidelines for Carcinogenic Risk Assessment* (USEPA 1996a) state that when the mode of action analysis based on available data indicates that "the carcinogenic response is secondary to another toxicity that has a threshold, the margin-of-exposure analysis performed for toxicity is the same as is done for a non-cancer endpoint, and an RfD for that toxicity may be considered in the cancer assessment".

This is the case for chloroform. That is, available evidence indicates that chloroform-induced carcinogenicity is secondary to cytotoxicity and regenerative hyperplasia, and that doses below the RfD do not result in cytolethality (and hence do not result in increased risk of cancer). Accordingly, the RfD developed above (1E-02 mg/kg-day) for protection against non-cancer effects (including cytolethality and regenerative hyperplasia) is also judged to be protective against increased risk of cancer.

4.2.4.3 Quantification of Cancer Risk from Inhalation Exposure

Only one study was located that is a candidate for quantification of cancer risks following inhalation exposure to chloroform (Nagano et al. 1998, also reported in Yamamoto et al. 1994 and Matsushima 1994). However, this study is not considered suitable for quantitative cancer dose-response modeling because the study had to rely on an increasing step-wise exposure protocol for male mice in the two highest exposure groups (30 and 90 ppm) in order to avoid lethality. This indicates that the 30 and 90 ppm doses exceed the maximum-tolerated dose (MTD), making it difficult to interpret these observations with respect to potential cancer effects at lower doses. Moreover, this study has not been published in full and the data are not available for detailed review.

In the absence of reliable quantitative inhalation cancer dose-response data for chloroform, risk of cancer from inhalation exposure was evaluated using a non-linear RfC-type approach, similar to that described above for oral exposure. Although data on the mode of chloroform action following inhalation exposure are less robust than for oral exposure, the available data suggest the mode of action is likely to be the same by both exposure routes. That is, both oral exposure and inhalation exposures of animals cause effects in the same target tissues (liver, kidney, nasal epithelium), and the histological and biochemical nature of the adverse effects observed in these tissues are the same in both cases. Further, in analogy with what is well-established for oral exposure, Templin et al. (1998) demonstrated that the inhalation exposure levels (30 and 90 ppm) that had been shown to produce renal tumors in male mice (Nagano et al. 1998) also produced evidence of renal cytotoxicity and cellular regeneration, while no evidence of renal cytotoxicity or cellular regeneration was noted in groups that did not display a

tumorigenic response (males at 5 ppm, females at all exposure levels). Based on this, EPA concluded that inhalation exposures that do not cause cytolethality and regenerative hyperplasia in liver or kidney will not cause increased risk of cancer (USEPA 2001a). That is, inhalation exposures below the RfC of 0.1 mg/m^3 (corresponding to an inhalation RfD of 0.03 mg/kg-day) do not result in cytolethality, and hence do not result in increased risk of cancer.

4.2.5 Discussion of Confidence

Available evidence is strong that chloroform can induce increased risk of cancer in animals. A large number of studies have been performed to investigate the hypothesis that this increased risk of cancer requires occurrence of cytotoxicty and regenerative cell proliferation. To date, the strength of this association is strong: there are no known cases of increased cancer incidence in animals that were not accompanied by clear evidence of cytotoxicity and regenerative hyperplasia in the target organ. While compelling, it is important to realize that a correlation of this type can never prove the proposed mode of action is correct, since the hypothesis could be disproved by only a single counter example. In addition, it is important to recall that even though the weight of evidence indicates that chloroform is not strongly mutagenic, there are a number of mutagenicity studies that yielded positive results, and it remains plausible that chloroform may have a weak potential to cause mutagenic effects under at least some dose conditions. On this basis, confidence in the cancer assessment is rated as medium. This confidence level may increase as further studies are performed on the mode of action.

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5. EXPOSURE ASSUMPTIONS

5.1 RELATIVE SOURCE CONTRIBUTION ANALYSIS

When an ambient water quality criterion is based on noncarcinogenic effects (as is the case for chloroform), anticipated exposures from non-occupational sources (e.g., food, air) are taken into account. As described in the 2000 Human Health Methodology (USEPA 2000b), EPA has, in the past, used a "subtraction" method to account for multiple sources of exposure to pollutants. However, EPA has also previously used a "percentage" method for the same purpose. In this approach, the percentage of total exposure typically accounted for by the exposure source for which the criterion is being determined, referred to as the relative source contribution (RSC), is applied to the RfD to determine the maximum amount of the RfD "apportioned" to that source. The underlying objective is to maintain total exposure below the RfD while generally avoiding an extremely low limit in a single medium that represents just a nominal fraction of the total exposure. To meet this objective, all proposed numeric limits lie between 80% and 20% of the RfD. EPA uses the Exposure Decision Tree in the 2000 Human Health Methodology (Figure 4-1 in USEPA 2000b) when deriving its AWQC values.

When more than one criterion is relevant to a particular chemical, as in the case of chloroform, apportioning the RfD via the percentage method of the Exposure Decision Tree is considered appropriate to ensure that the combination of criteria, and thus the potential for resulting exposures, do not exceed the RfD. In following the Exposure Decision Tree, it is necessary that adequate data exist for the relevant sources/pathways of exposure if one is to avoid using default procedures. The adequacy of data is a professional judgement for each individual chemical of concern, but EPA recommends that the minimum acceptable data are exposure distributions that can be used to determine, with an acceptable 95% confidence interval, the central tendency and high-end exposure levels for each source. If there are not sufficient data/information to characterize exposure, particularly to the media of concern, EPA intends to use the "default" assumption of 20% of the RfD when deriving or revising the RfD.

5.1.1 Population of Concern

For chloroform, the population of concern for setting national criteria is assumed to be the adults of the general population of the United States. Based on a review of CYP2E1 levels in the fetus and in children, EPA concluded that there is no basis to conclude that the mode of action of chloroform would differ between children and adults, and that

neither the fetus nor the child appears to more sensitive than adults based on level of CYP2E1 activity (USEPA 2001a).

5.1.2 Overview of Potential for Exposure

Humans may be exposed to chloroform from a number of different media and by several different exposure routes. Figure 5-1 is a conceptual model that summarizes these exposure sources and routes.

5.1.2.1 Ambient Water

The principal pathways by which humans may be exposed to chloroform in ambient water include ingestion of the water as drinking water and ingestion of fish and other aquatic organisms collected from the water.

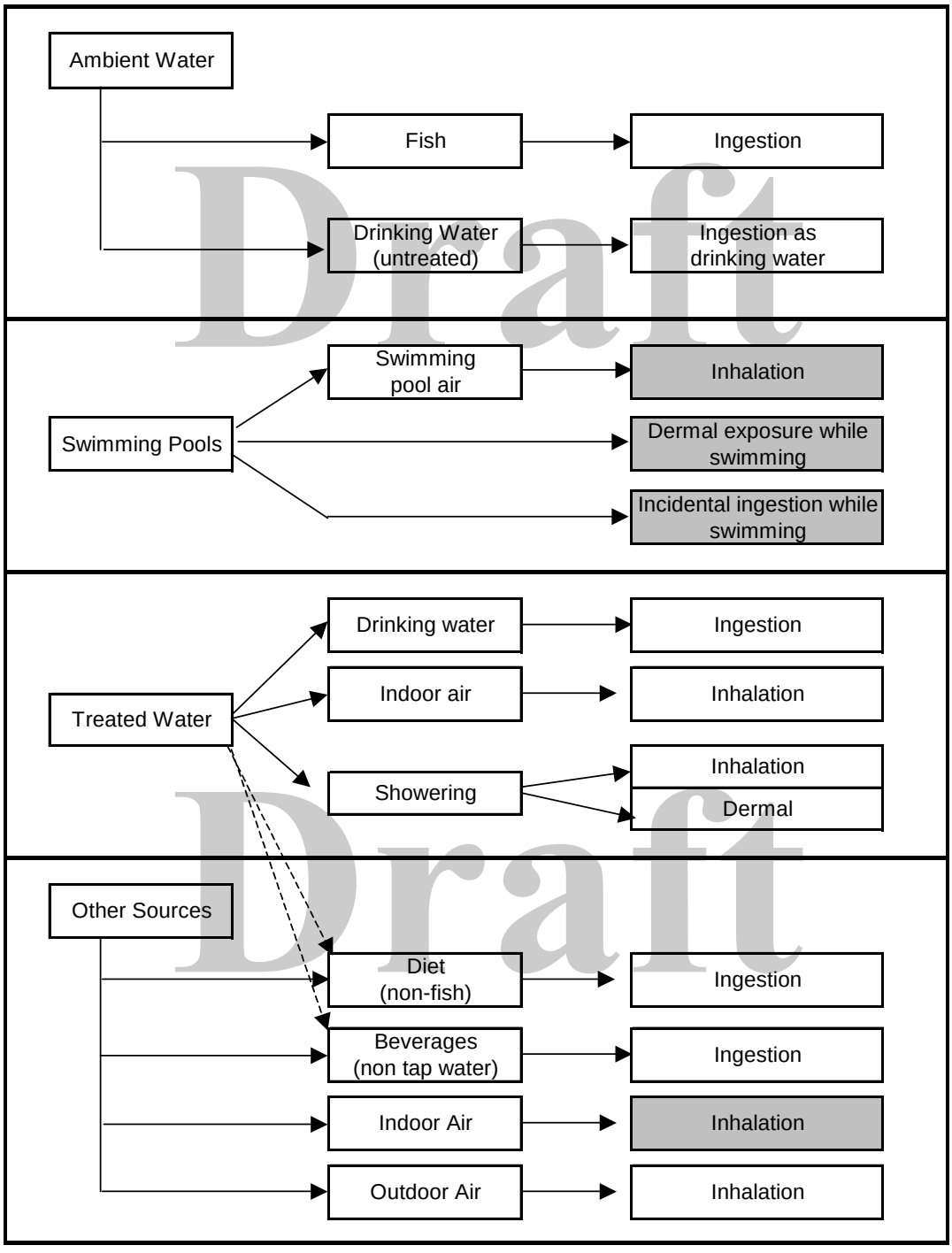
Exposure to chloroform in ambient water may also occur by incidental ingestion during recreational activities, but this pathway is generally so minor that it is not included in the derivation of the AWQC (USEPA 2000b). Likewise, dermal contact during recreational exposures to ambient water are likely to be so minor that they are not included.

5.1.2.2 Treated Water

When water is treated with a strong oxidant such as chlorine, chlorine dioxide, or chloramine to kill potentially pathogenic organisms that may be present in the water, chlorine is formed as a disinfection byproduct (DBP) by oxidation of organic matter that is present in the water. Residents in homes supplied with treated drinking water may be exposed to chloroform by three main routes: ingestion, inhalation of chloroform vapors that have been released from the water into indoor air, and dermal contact with the water while showering or bathing.

Exposure to chloroform may also occur during swimming in a pool that is disinfected with chlorine. Exposure during swimming may occur by ingestion, inhalation, and dermal pathways. However, the frequency of such exposures is relatively small for the general population, and estimated exposures via these pathways are small compared to other pathways (USEPA 2000b). Therefore, exposures during swimming are not included in the derivation of the AWQC.

FIGURE 5-1 EXPOSURE PATHWAYS FOR CHLOROFORM



5.1.2.3 Non-Water Sources

Chloroform may also be found in various dietary items, and this can contribute to oral exposure of humans. It should be noted that some of the chloroform present in dietary items may be attributable to chloroform that was present in treated water used in the preparation of the food as well as from the use of chlorine disinfectants in the processing of foods or the cleaning of food contact surfaces. Chloroform may also be released to indoor and outdoor air from a variety of industrial sources and from consumer products, including household bleach, that contain chloroform, and this can lead to inhalation exposure of humans.

5.1.3 Estimates of Exposure from Non-Ambient Water Sources

EPA has published an extensive review of human exposure to chloroform from a variety of environmental media (USEPA 2001b). Information presented below on concentration levels and exposures from various non-ambient water related pathways are taken from that report. It should be noted that all exposure estimates for these exposure pathways are based on absorbed doses and are intended to estimate median exposures for the general population.

5.1.3.1 Exposure from Treated Drinking Water

Ingestion Exposure

The basic equation for calculating the average daily absorbed dose (ADD) of chloroform from ingestion of treated water as drinking water is as follows:

$$ADD(\text{ingestion of treated water}) = C_{TW} \times IR_{TW} \times AF_o / BW$$

Input values and the resultant ADD value are summarized below:

Parameter	Description	Value	Units	Source
C_{TW}	Mean concentration of chloroform in treated drinking water	24	µg/L	USEPA 2001b
IR_{TW}/BW	Average ingestion rate of treated water	0.019	L/kg-day	USEPA 2001b
AF_o	Oral absorption fraction for chloroform	1.0	--	USEPA 2001b
ADD	Average daily absorbed dose from ingestion of treated drinking water	0.46	µg/kg-day	Calculated from inputs

General Inhalation Exposure

The basic equation for calculating the average daily absorbed dose of chloroform from inhalation of chloroform that is released from treated water into general indoor air is as follows:

$$ADD(\text{inhalation of vapors from treated water}) = C(air)_{general} \times BR \times ET \times AF_i / BW$$

Data on the concentration of chloroform in general indoor air indicate that the average value is about 3.0 µg/m^3 (USEPA 2001b). This measurement includes chloroform from all sources and does not distinguish the amount of chloroform due to releases from water from that which is due to releases from other sources. However, a screening level calculation of the fraction due to water is possible, as follows.

The release of radon from water to indoor air has been well-studied, and the average transfer factor for radon from water to indoor air is about 0.1 L/m^3 (USEPA 2001c). Based on physical-chemical properties, the release of chloroform from water into air is expected to be about 75% that of radon (McKone 1987). Thus, the predicted average increment in general indoor air due to releases from water is as follows:

$$C(air)_{general} = 24\ \mu g/L \times 0.075\ L/m^3 = 1.8\ \mu g/m^3$$

Adding the average concentration in outdoor air (about 1.6 µg/m^3) (USEPA 2001b) to the water-related increment (1.8 µg/m^3) yields a predicted total indoor air mean concentration of about 3.4 µg/m^3, a value that is slightly higher than the measured mean value (3.0 µg/m^3) (USEPA 2001b). This calculation indicates that measured levels of chloroform in indoor air are likely due mainly to releases from treated water plus the contribution from outdoor air, and releases from other (non-water) indoor sources are likely to be so small that they can be ignored.

Based on this, input values and the resultant ADD value are summarized below:

Parameter	Description	Value	Units	Source
$C(air)_{general}$	Mean concentration of chloroform in general indoor air	3.0	µg/m^3	USEPA 2001b
BR	Average breathing rate while indoors	0.67	m^3/hour	USEPA 2001b
ET	Average exposure time indoors	24	hours/day	USEPA 2001b
AF_i	Inhalation absorption fraction	0.63	--	USEPA 2001b
BW	Mean body weight	70	kg	USEPA 2000b
ADD	Average daily absorbed dose from breathing chloroform in indoor air	0.43	µg/kg-day	Calculated from inputs

Inhalation Exposure While Showering

The basic equation for calculating the average daily absorbed dose of chloroform from inhalation of chloroform in bathroom air that is released from treated water during showering is as follows:

$$ADD(\text{inhalation of vapors while showering}) = C(air)_{shower} \times BR \times ET \times AF_i / BW$$

Input values and the resultant ADD value are summarized below:

Parameter	Description	Value	Units	Source
$C(air)_{shower}$	Mean concentration of chloroform in air during showering	190	µg/m^3	USEPA 2001b
BR	Average breathing rate while showering	0.66	m^3/hour	USEPA 2001b
ET	Average exposure time during and after showering	0.12	hours/day	USEPA 2001b
AF_i	Inhalation absorption fraction	0.63	--	USEPA 2001b
BW	Mean body weight	70	kg	USEPA 2000b
ADD	Average daily absorbed dose from breathing chloroform in shower air	0.14	µg/kg-day	Calculated from inputs

Dermal Exposure While Showering

The basic equation for calculating the average daily absorbed dose of chloroform from dermal contact with treated water used for showering or bathing is as follows:

$$ADD(\text{dermal contact with treated water}) = C_{TW} \times DA \times ED \times SAR$$

Input values and the resultant ADD value are summarized below:

Parameter	Description	Value	Units	Source
C_{TW}	Mean concentration of chloroform in treated water	24	µg/L	USEPA 2001b
DA	Dermal absorption rate from water	3.52E-06	µg per µg/L per cm^2-min	USEPA 2001b
ED	Showering exposure duration	5	min/day	USEPA 2001b
SAR	Surface area to body weight ratio	290	cm^2/kg	USEPA 2001b
ADD	Average daily absorbed dose from dermal contact with treated water	0.12	µg/kg-d	Calculated from inputs

5.1.3.2 Exposures from Outdoor Air

The basic equation for calculating the average daily absorbed dose of chloroform from inhalation of chloroform in outdoor air is as follows:

$$ADD(\text{inhalation in outdoor air}) = C(air)_{outdoor} \times BR \times ET \times AF_i / BW$$

Concentration values of chloroform in outdoor air vary substantially depending on the location of measurement. Based on data tabulated in USEPA (2001b), the mean concentration for locations in the United States is about 1.6 µg/m^3. Assuming an average outdoor exposure of 2 hours/day (USEPA 1997b), the resultant ADD value is as summarized below:

Parameter	Description	Value	Units	Source
$C(air)_{outdoor}$	Mean concentration of chloroform in outdoor air (United States)	1.6	µg/m^3	USEPA 2001b
BR	Average breathing rate	0.67	m^3/hour	USEPA 2001b
ET	Average exposure time outdoors	2	hours/day	USEPA 1997b
AF_i	Inhalation absorption fraction	0.63	--	USEPA 2001b
BW	Mean body weight	70	kg	USEPA 2000b
ADD	Average daily absorbed dose from breathing chloroform in indoor air	0.019	µg/kg-day	Calculated from inputs

5.1.3.3 Dietary Exposures

USEPA (2001b) summarized data on the concentration levels and estimated exposure levels to chloroform in a wide variety of dietary items. These data are summarized in Table 5-1. As shown, the estimated absorbed dose of chloroform from all dietary sources (excluding freshwater and estuarine fish) is about 0.46 µg/kg-day, with dairy products and grain contributing the largest intakes.

5.1.3.4 Total Non-Ambient Water Exposures

The total exposure from all non-ambient water sources is summarized in Table 5-2. As seen, the estimated total average daily absorbed dose is about 1.6 µg/kg-day if exposures from treated drinking water are included, and about 0.5 µg/kg-day if exposures from treated water are excluded.

5.1.4 Estimates of Exposure from Ambient Water Sources

5.1.4.1 Ingestion of Ambient Water

USEPA (2001b) summarizes data from a number of studies on chloroform levels in ambient surface water bodies (mainly streams and rivers). Average levels typically ranged from 0.2 to 5 µg/L. However, nearly all of these data (15 out of 16 observations) are from locations outside of the United States (mainly Finland). The only study with data from within the US (Sheldon and Hites 1978) reported that chloroform levels in water samples from the Delaware River were below the detection limit. However, the detection limit was not reported. Thus, it is concluded that data on chloroform levels in ambient waters of the US are too limited to allow reliable quantitation of exposure through ingestion.

TABLE 5-1. DIETARY EXPOSURES TO CHLOROFORM

Category	Type	C ug/g	IR g/kg-day	ADD ug/kg-day
Fruits/Vegetables	Fruits	0.010	1.6	0.016
	Exposed veg	0.025	1.2	0.030
	Protected veg	0.019	0	0.000
	Root veg.	0.016	0.78	0.012
Meat/Dairy	Beef	0.090	0.54	0.049
	Pork	0.038	0.07	0.003
	Lamb	0.060	0.0056	0.0003
	Sausage	0.017	0.12	0.002
	Poultry	0.038	0.30	0.011
	Dairy	0.079	2.60	0.205
Grain	All	0.045	2.6	0.117
Marine Fish	All	0.052	0.154	0.008
TOTAL				0.454

All data are from USEPA (2001b)

TABLE 5-2. SUMMARY OF NON-AMBIENT WATER EXPOSURES

Source	Pathway	ADD ug/kg-day
Treated Water	Ingestion	0.46
	General Inhal	0.43
	Shower Inhal	0.14
	Shower Dermal	0.12
Ambient Air	Outdoor Inhal	0.02
Diet	Fruits	0.02
	Veg	0.04
	Meat	0.07
	Dairy	0.21
	Grain	0.12
	Marine fish	0.01
Total (Including treated water)		1.62
Total (Excluding treated water)		0.47

5.1.4.2 Ingestion of Freshwater and Estuarine Fish

No data were located on the concentration of chloroform in freshwater or estuarine fish or other aquatic species. Thus, data are not sufficient to quantitate exposure from ingestion of fish from ambient water sources.

5.2 EXPOSURE DATA ADEQUACY AND UNCERTAINTY ESTIMATES

As noted above, the main source of human exposure to chloroform is through the use of treated (disinfected) water for drinking and other indoor uses. Data on the level of chloroform in treated water are extensive, and all public drinking water systems are subject to national regulations that limit the levels of chloroform and other trihalomethanes that may be present in treated water. Human exposure to chloroform in treated water is highly variable, depending both on human behavioral factors (amount of water ingested, time spent in the shower, time spent at home, etc.), and on house construction variables (size of the home, ventilation rate, water use rate, etc.). However, reliable data are available to characterize all of these exposure variables, and hence estimates of human exposure to chloroform from treated water are reasonably certain.

Data on chloroform levels in the diet are less extensive than for water, but are sufficient to establish that exposure through the diet is relatively small compared to the sum of all exposure pathways (ingestion, inhalation, dermal) related to use of treated water.

Data on chloroform levels in ambient water are very limited, and no data were located on chloroform levels in fish from freshwater or estuarine sources. Thus, data are inadequate to allow reliable quantitation of typical exposure levels from ambient water sources.

5.3 RSC ESTIMATES/ALLOCATION OF THE RfD

RSC for Exposure from Ingestion of Fish Only

In the case of an individual who is exposed to ambient water only by ingestion of freshwater/estuarine fish, the equation for calculating the RSC is as follows:

$$RSC = ADD(freshwater\ fish) / ADD(total)$$

As discussed above, when total exposure includes exposures to treated water, the total ADD is about 1.6 µg/kg-day (see Table 5-2). The precise fraction of this total dose that is contributed by ingestion of freshwater or estuarine fish is unknown because no data

could be located on chloroform levels in freshwater fish. In accord with the Exposure Decision Tree approach described in Figure 4-1 of USEPA (2000b), when data are not available to derive reliable quantitative estimates of all relevant exposure distributions, a default "floor" value of 20% is recommended for the RSC (Box 8B). Based on this guidance, 20% is selected as the RSC for this exposure scenario.

RSC for Exposure from Ingestion of Fish and Use of Ambient Water for Drinking

In the case on an individual who is exposed to ambient water both by ingestion of freshwater/estuarine fish and by use of the ambient water for drinking water, it is assumed that the water used for ingestion is not treated[1]. In this case, the equation for calculating the RSC is as follows:

RSC = ADD(freshwater fish + Ingestion of ambient water) / ADD(total)

As discussed above, when exposure does not include treated water, the total ADD about 0.5 µg/kg-day (see Table 5-2). The precise fraction of this total dose that is contributed by ingestion of freshwater or estuarine fish is unknown because no data could be located on chloroform levels in freshwater fish. Likewise, the fraction of the dose contributed by ingestion of ambient water is also unknown, since no reliable data could be located on chloroform levels in ambient surface waters of the United States. In accord with the Exposure Decision Tree approach described in Figure 4-1 of USEPA (2000b), when data are not available to derive reliable quantitative estimates of all relevant exposure distributions, a default "floor" value of 20% is recommended for the RSC (Box 8B). Based on this guidance, 20% is selected as the RSC for this exposure scenario.

5.4 EXPOSURE ASSUMPTIONS FOR AMBIENT WATER

As noted above, humans may be exposed to chloroform in ambient water by ingestion as drinking water and by ingestion of fish or other aquatic food items collected from the ambient water body.

In order to ensure that the AWQC value is protective, the combinations of exposure parameter values selected for quantifying exposure to chloroform from these pathways are intended to estimate exposures of individuals who are at the upper end of the range of possible exposure levels. This is achieved by selecting one or two of the key variables in each exposure equation and setting them at their high-end values (e.g., 90th

[1] If the ambient water were treated before use in the house, exposure would be evaluated as described for Scenario 1.

to 95th percentile), while other values are held at their median or average value. The selection of these exposure parameters is described below.

5.4.1 Exposure from Ambient Water Used for Drinking

The basic equation for calculating the average daily dose of chloroform from ingestion of ambient water that contains a concentration of C_{AW} (µg/L) as drinking water is as follows:

$$Average\ Daily\ Intake(water) = C_{AW} \times DI_{AW} / BW$$

Exposure parameters needed to calculate the daily intake from this pathway are summarized below:

Parameter	Description	Value	Units	Comment	Source
DI_{AW}	daily intake rate of ambient water	2	L/day	High end value (AWQC default)	USEPA 2000b
BW	Body weight	70	kg	Typical value (AWQC default)	USEPA 2000b

5.4.2 Exposures from Fish Ingestion

The basic equation for calculating the average daily dose of chloroform from ingestion of fish collected from an ambient freshwater or estuarine water body that contains a concentration of C_{AW} (µg/L) is as follows:

$$Average\ Daily\ Intake(fish) = C_{AW} \times BAF \times FI / BW$$

Because both intake rates and BAFs vary among different types of fish, the basic equation is stratified into three trophic levels, as follows:

$$Average\ Daily\ Intake(fish) = C_{AW} \times \sum_{3} (BAF_{TL\,i} \times FI_{TL\,i} / BW)$$

Exposure parameters needed to calculate the ADD from this pathway are summarized below:

Parameter	Description	Value	Units	Comments	Source
$BAF_{TL\ i}$	Bioaccumulation factor for trophic level i	See Section 6	µg/kg tissue per µg/L	Best estimate based on available data	See Section 6
$FI_{TL\ i}$	Ingestion rate for fish from trophic level i	TL2 =3.8 TL3 = 8.0 TL4 = 5.7	g tissue/day	High end (90th percentile) values (AWQC defaults)	USEPA 2000b
BW	Body weight	70	kg	Typical value (AWQC default)	USEPA 2000b

6. BIOACCUMULATION FACTORS

This section describes the procedures and data sources used to calculate the bioaccumulation factors (BAFs) used for deriving an AWQC for chloroform. Details and the scientific basis of EPA's recommended methodology for deriving BAFs are described in USEPA (2000b) and USEPA (2003).

When determining BAFs for use in deriving AWQC for nonionic organic chemicals, two general steps are required. The first step consists of calculating baseline BAFs for organisms at appropriate trophic levels using available field and laboratory studies of the bioaccumulation or bioconcentration of the chemical of interest. Since baseline BAFs are normalized by important factors shown to affect bioaccumulation (e.g., the lipid content of aquatic organisms on which they are based, the freely dissolved concentration of the chemical in water), they are more generally applicable than BAFs not adjusted for these factors. Once baseline BAFs have been calculated for the appropriate trophic levels, the second step involves adjusting the baseline BAFs to reflect the expected conditions at the sites that are applicable to the AWQC (e.g., lipid content of consumed organisms and the freely dissolved fraction of the chemical in the site water). Application of both of these steps to the derivation of BAFs for chloroform is described below.

6.1 BASELINE BAFs

In EPA's framework for deriving national BAFs, several different procedures are presented by which to derive national BAFs, the applicability of which depends on the properties of the chemical of interest (USEPA 2000b, 2003). According to the guidance provided, nonionic chemicals with log K_{ow} values less than 4.0 should be classified as exhibiting low hydrophobicity. The log K_{ow} for chloroform is less than 2, hence it is considered to have low hydrophobicity. Based on the hydrophobicity of chloroform, national BAFs for chloroform are calculated according to Procedure 3, as discussed in detail in USEPA (2000b, 2003). Within Procedure 3, two methods are recommended for determining the baseline BAFs, depending on the type of bioaccumulation data available. The data preference for deriving a BAFs for non-polar organics with low hydrophobicity is (in order of preference):

Method 1: calculation of a baseline BAF from a reliable field-measured BAF or laboratory-measured BCF;

Method 2: calculation of a baseline BAF from the chemical's octanol-water partition coefficient (K_{ow}).

For nonionic organic chemicals that exhibit low hydrophobicity, available information indicates that non-aqueous exposure to these chemicals is not likely to be important in determining chemical bioaccumulation in aquatic organisms (USEPA, 2000b). For this group of chemicals, Baseline BAFs derived from laboratory-measured BCFs and K_{ow} do not require adjustment with food-chain multipliers (FCMs) for determining the national BAFs.

Fish consumption rates determined from the USDA's Continuing Survey of Food Intakes by Individuals (CSFII) indicate that on a national, average per capita basis, individuals in the United States consume significant quantities of fish and shellfish at trophic levels two (e.g., clams, oysters), three (e.g., crab, shrimp, flounder) and four (e.g., trout, pike, certain catfish species) (USEPA 1998e). Therefore, the national AWQC for chloroform requires that BAFs be derived to reflect bioaccumulation in aquatic organisms at each of these three trophic levels.

6.1.1 Summary of Field-derived BAF and Laboratory-measured BCF Data

A total of 15 literature reports were located that contained data potentially useful in the derivation of BAFs for chloroform. Each was reviewed to determine the relevance and adequacy of the data reported for application in deriving Baseline BAFs. Table 6-1 summarizes the results of this review. As seen, only one study (Anderson and Lusty 1980) was judged to have potentially useful data on which to base chloroform BAFs in freshwater/estuarine species.

Table 6-1 summarizes the data from Anderson and Lusty (1980). Laboratory BCF values were not reported by the authors, but data (water and tissue concentrations) necessary for calculating them were provided. Rainbow trout, bluegill sunfish, largemouth bass, and channel catfish were exposed to aerated water collected from the Columbia River as part of a 24-hour static exposure study. Concentrations of chloroform were measured in whole body tissue of each fish and in the exposure water. A semi steady-state condition appeared to be reached within 24 hours for the bluegill, trout and bass, but not the catfish. Maximum concentrations of chloroform in trout and bass tissues were reached in 4 hours. Measured concentrations of chloroform in fish tissue were only slightly greater than in the exposure water, indicating that chloroform has a relatively low tendency to bioconcentrate in fish tissue.

Further examination of the study by Anderson and Lusty (1980) revealed that the lipid content of the fish used in the study was not reported. According to the data quality considerations provided in USEPA (2003), the lipid content of study organisms must be measured or reasonably estimated in order for a laboratory-measured BCF to be

TABLE 6-1 SUMMARY OF STUDIES ON CHLOROFORM UPTAKE BY FISH

Reference	Study Type	Water Type or Location	Habitat Type	Species Name	Common Name	Trophic Level	Data Evaluation	Comments on Relevance and Data Adequacy
Baumann-Ofstad et al. 1981. Science of the Total Environment. 20: 205-215.	Field	Iddefjord, Norway	Marine	species not specified	eel	4	Inadequate	Article lacks detail on the measurement of chloroform in water. Only a range of water concentrations is given - 0.3 to 13 ug/L.
Anderson D.R. and E.B. Lusty. 1980. Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Freshwater Fish. U.S. Nuclear Regulatory Commission	Lab	Aerated Columbia River Water	NA	Lepomis macrochirus	bluegill sunfish	3	Accept	24-hour static exposure. A semi steady-state condition appeared to be reached withing 24 hrs for bluegill, trout and bass, but not catfish. Maximum concentrations of chloroform in trout and bass were reached in 4 hrs. Chloroform in water was measured.
				Oncorhynchus mykiss	rainbow trout	4	Accept	
				Micropterus salmoides	largemouth bass	4	Accept	
				Ictalurus punctatus	channel catfish	4	Inadequate	
Diamond M.L. 1994. Journal of Great Lakes Research 20(4): 643-666.	Model	Predicted conditions in Bay of Quinte, Lake Ontario	Lentic		plankton	1-2	Inadequate	Tissue and water chloroform values are estimates generated using a fugacity model. Water data were modeled using input from water chloroform values reported in Comba and Kaiser (1984), J. Gr. Lakes Res. 19:375. Authors state that uncertainty in the estimates could vary by as much as 10-fold.
					sculpin	3	Inadequate	
				Perca flavescens	Y-of-Y perch	2-3	Inadequate	
				Perca flavescens	perch	3	Inadequate	
				Stizostedion vitreum	walleye	4	Inadequate	
Pearson, C.R. and G. McConnell. 1975. Proc. R. Soc. Lond. 189: 305-332.	Field	Liverpool Bay, United Kingdom	Marine		zooplankton	2	Inadequate	Article lacks detail regarding the measurement of chloroform in water. Only a range of water concentrations is given - 0.01 to 1 ug/L.
				Mytilus edulis	mussel	2	Inadequate	
				Cerastoderma edule	cockle	2	Inadequate	
				Cancer pagurus	crab	3	Inadequate	
				Platycthys flesus	flounder	4	Inadequate	
				Scomber scombrus	mackerel	4	Inadequate	
Roose, P. and U.A.Th. Brinkman. 1998. Journal of Chromatography A. 799: 233-248.	Field	Belgian Continental Shelf	Marine	Limanda limanda	dab	4	Inadequate	The concentration of chloroform in water was not measured with the fish samples. The authors, however, do refer to an article by DeWulf and Van Langenhove (1995). Internal. J. Environ. Anal. Chem. 61:35 who did measure water chloroform concentrations in water in proximity to the biota sample sites, and apparently at a similar date.
				Merlangius merlangus	whiting	3	Inadequate	

Reference	Study Type	Water Type or Location	Habitat Type	Species Name	Common Name	Trophic Level	Data Evaluation	Comments on Relevance and Data Adequacy
Toussaint et al. 2001. Environmental Health Perspectives. 109(1):35-40	Lab	Processed well water	NA	Oryzias latipes	Japanese medaka	2	Inadequate	9 month flow-through exposure. Chlorform was not measured in edible or whole-body tissue.
Voss, R.H. 1983. Environmental Science Technology. 17(9): 530-537.	Lab	Bleached Kraft Mill Effluents	NA				Inadequate	No tissue data reported.
Yoshida, K. 1993. Chemosphere. 27(4): 621-630.	Model		NA				Inadequate	No original tissue or water data provided. Model predicted BCF of 15.7 could be calculated based on fish and drinking water values reported in Table 6 - Calculated exposure concentrations and doses for humans.
Barrows et al. 1980. Toxic Chemicals. pp 379-392.	Lab	Well water		Lepomis macrochirus	bluegill sunfish	3	Inadequate	14-day flow-through exposure. Parent compound was not quantified in biological tissue. Half-life of chloroform in tissue estimated to be less than 1 day.
Darnerud et al. 1989. Journal of Toxicology and Environmental Health 26: 209-221.	Lab	Aerated City of Uppsala, Sweden tapwater	NA	Oncorhynchus mykiss	rainbow trout	4	Inadequate	8-hr static exposure. Parent compound was not quantified in edible biological tissue. Contrary to rodents, the data indicated that most chloroform binding in liver and kidney was not irreversibly bound in the form of electrophilic metabolites.
Veith et al. 1980. Aquatic Toxicology. 116-128	Lab	Well water	NA	Lepomis macrochirus	bluegill sunfish	3	Inadequate	Data is the same as reported in Barrows et al. (1980), reference #9, this spreadsheet
Hendriks, A.J. 1995. Ecotoxicology and Environmental Safety. 32:103-130.	Model		NA	Oncorhynchus mykiss	rainbow trout	4	Inadequate	Tissue chloroform concentrations were estimated using a model of lethal response doses from Hodson et al. (1984). Environ. Toxicol. Chem. 3:243-254 and Hodson (1985). J. Appl. Toxicol. 5:220-226. No corresponding values in water are provided.
Hiatt et al. 1981. Analytical Chemistry. 53:1541-1543.	Lab		NA		Unspecified fish species		Inadequate	Paper described an analytical methods development. Chloroform in tissue, sediment, and water were given as percent recoveries from a matrix spike (25 ppb).
Paasivirta et al. 1983. Chemosphere. 12(2):239-252	Field	Lakes in Central Finland	Lentic	Rutilus rutilus	roach	3	Inadequate	Chloroform was not measured in water, nor was it in roach at levels sufficient for quantification.

Reference	Study Type	Water Type or Location	Habitat Type	Species Name	Common Name	Trophic Level	Data Evaluation	Comments on Relevance and Data Adequacy
Young et al. 1983. Chapter 60 In: Water Chlorination, Book 2, Vol. 4, pp. 871-884.	Field	San Pedro Bay, Los Angeles, CA	Marine	Sicyonia ingentis	ridgeback prawn	3	Inadequate	Tissue chloroform concentrations were reported as less than values, with fish liver and shrimp muscle values (< 2 ug/kg ww) below the analyical quantification limit in tissue of 10 ug/kg ww. Representative fish tissue values were for a non-edible tissue (liver). Biota samples were collected up-coast of the municipal effluent outfall where chloroform in water was measured.
				Genyonemus lineatus	white croaker	3	Inadequate	
				Scorpaena guttata	Calfornia scorpion fish	4	Inadequate	
				Microstomus pacificus	Dover sole	4	Inadequate	
				Citharichthys sordidus	Pacific sanddab	4	Inadequate	

TABLE 6-2 SUMMARY OF RELIABLE BCF DATA

Citation	Habitat Type	Exposed Organism	Scientific Name	Trophic Level	C(water) (mg/L)	C(tissue) (mg/kg)	BCF_T^t (L/kg)	f_R (a)	BAF_R^{fd} (b)	Species BAF_R^{fd}	Trophic Level Baseline BAF_R^{fd}
Anderson and Lusty (1980)	Freshwater	rainbow trout	*Oncorhynchus mykiss*	4	1	3.34	3.34	0.051	45.9	91.7	91.7
					1	10.35	10.35	0.051	183.1		

(a) Lipid content of fish tissue was not reported in the study; used species-mean value for rainbow trout from USEPA (2003).
(b) Where f_{fd} is assumed to be 1.0.

acceptable for deriving a national BAF. Although the lipid contents of the organisms studied by Anderson and Lusty (1980) are not available, USEPA (2003) provides species-specific average lipid values for fish species commonly consumed by the U.S. population. This compilation of lipid values includes an average lipid percentage for rainbow trout (5.1%), but not for the other species used in the Anderson and Lusty study. Therefore, a baseline BAF for trophic level 4 will be derived using this average percent lipid value for rainbow trout from USEPA (2003) and the rainbow trout data from Anderson and Lusty (1980).

Due to their low hydrophobicity (i.e., log K_{ow} <4.0), nonionic organic chemicals to which Procedure 3 is applied are expected to remain almost entirely in the freely dissolved from in waters containing dissolved and particulate organic carbon concentrations typical of laboratory BCF studies (USEPA 2000b). Therefore, the freely dissolved fraction is usually assumed to be 1.0. For the calculation of a baseline BAF from the BCF study of Anderson and Lusty (1980), the f_{fd} is assumed to be 1.0.

6.1.2 Derivation of Baseline BAFs (BAF_R^{fd}s)

According to the data preference hierarchy specified above, method 1 will be used for determining the baseline BAFs for trophic level 4. In accordance with this method, each laboratory-measured BCF (expressed as total concentration in tissue divided by total concentration in water) was adjusted to a baseline BAF (expressed as lipid-normalized concentration in tissue divided by freely-dissolved concentration in water) using Equation 6.1.1 below:

$$Baseline\ BAF_{\ell}^{fd} = \left[\frac{BCF_T^t}{f_{fd}} - 1\right] - \frac{1}{f_{\ell}} \qquad \text{[Equation 6.1.1]}$$

where:

Baseline BAF_R^{fd}	=	BAF expressed on a freely-dissolved and lipid-normalized basis (L/kg lipid)
BCF_T^t	=	laboratory BCF based on total concentration of chemical in tissue and water (L/kg tissue)
f_R	=	fraction of the tissue that is lipid (kg/kg)
f_{fd}	=	fraction of the total chemical that is freely-dissolved in the water of the BAF study

Based on these inputs, the baseline BAF calculations are as shown in the right side of Table 6-2. For trophic level four, based on a BCF derived for rainbow trout, the baseline BAF is 91.7 L/kg-lipid.

Because no acceptable measured BAF or BCF data were located for trophic levels 2 and 3, Baseline BAFs for these trophic levels are estimated by method 2 within Procedure 3 of the BAF Derivation Framework. The 2000 Human Health Methodology recommends that individual Baseline BAFs be calculated using as many of the methods in Procedure 3 as possible, and the selection of the final baseline BAF for each trophic level be determined from the individual baseline BAF_R^{fd}s by considering the data preference hierarchy defined in Procedure 3 and uncertainty in the data. Therefore, method 2 will also be used to calculate a baseline BAF for trophic level 4. In accordance with this method, baseline BAFs for trophic levels 2, 3, and 4 are calculated from the K_{ow} for chloroform using Equation 6.1.2 below:

$$Baseline\ BAF_{\ell}^{fd} = K_{OW} \qquad \text{[Equation 6.1.2]}$$

where:

Baseline BAF_R^{fd} = BCF expressed on a freely-dissolved and lipid-normalized basis

K_{ow} = octanol-water partition coefficient

The log K_{ow} for chloroform is 1.97 (see Section 2). As mentioned previously, food-chain multipliers (FCMs) are not applicable for chemicals with low hydrophobicity (USEPA (2000b). Based on these inputs, the baseline BAFs for trophic level 2, 3, and 4 are calculated as follows:

Baseline BAF_R^{fd} (TL2) = (K_{ow})
= $(10^{1.97})$
= 93.3 L/kg-lipid

Baseline BAF_R^{fd} (TL3) = (K_{ow})
= $(10^{1.97})$
= 93.3 L/kg-lipid

Baseline BAF_R^{fd} (TL4) = (K_{ow})
= $(10^{1.97})$
= 93.3 L/kg-lipid

Due to lack of appropriate field-measured BAFs and laboratory-measured BCFs, there is only one Baseline BAF_R^{fd} value for trophic levels 2 and 3. Therefore, the final Baseline BAF_R^{fd} for trophic level 2 is 93.3 L/kg and for trophic level 3 is 93.3 L/kg. For trophic level 4, there was one laboratory-measured BCF value available from which a Baseline BAF_R^{fd} of 91.7 L/kg was derived. Uncertainties associated with this value include the fraction lipid value, which was estimated from species mean data rather than from study-specific fish lipid content determination, and the f_{fd}, which was assumed to be 1.0 based on general characteristics of chemicals with low hydrophobicity rather than from measured POC and DOC values. The Baseline BAF_R^{fd}s for trophic level 4 calculated by methods 1 and 2 are essentially the same. Because both trophic level 4 Baseline BAFs have moderate to low uncertainty associated with them, the Baseline BAF_R^{fd} calculated by method 1 (from laboratory-measured BCF) will be used as the final Baseline BAF_R^{fd} in accordance with the data preference hierarchy outlined for Procedure 3 (Section 6.1; USEPA 2000b).

6.2 National BAFs

After the derivation of trophic level-specific baseline BAFs for chloroform (described in the previous section), the next step is to calculate BAFs that will be used in the derivation of AWQC. This step is necessary to adjust the baseline BAFs to conditions that are expected to affect the bioavailability of chloroform at the sites applicable to the AWQC. Derivation of the National BAFs requires information on: (1) the baseline BAF at appropriate trophic levels, (2) the percent lipid of the aquatic organisms consumed by humans at the site(s) of interest (trophic-level specific), and (3) the freely dissolved fraction of the chemical in ambient water at the site(s) of interest. For each trophic level, the equation for deriving a BAF to use in deriving AWQC is:

$$National\ BAF_{(TL\ n)} = [(Baseline\ BAF_R^{fd})_{TL\ n} \times (f_R)_{TL\ n} + 1] \times (f_{fd}) \qquad \text{[Equation 6.2.1]}$$

where:

National $BAF_{(TL\ n)}$ = BAF at trophic level "n" used to derive AWQC based on site conditions for lipid content of consumed aquatic organisms for trophic level "n" and the freely dissolved fraction in the site water

$(Baseline\ BAF_R^{fd})_{TLn}$ = BAF expressed on a freely dissolved and lipid-normalized basis for trophic level "n"

$(f_R)_{TLn}$ = fraction lipid of aquatic species consumed at trophic level "n"

f_{fd} = fraction of the total chemical in water that is freely dissolved

Each of the components of the National BAF equation are discussed below.

6.2.1 Baseline BAFs (Baseline BAF_R^{fd})

The derivation of baseline BAFs at specific trophic levels is described in Section 6.1. For chloroform, a baseline BAF of 93.3 L/kg was derived from K_{ow} for aquatic organisms in trophic levels 2 and 3. For organisms in trophic level 4, a baseline BAF of 140.2 L/kg was derived from a laboratory-measured BCF and a baseline BAF of 93.3 L/kg was derived from K_{ow}.

6.2.2 Lipid Content of Consumed Aquatic Species

Accumulation of non-polar organic chemicals in aquatic organisms has repeatedly been shown to be a function of lipid content (e.g., Mackay 1982, Connolly and Pedersen 1988, Thomann 1989). Therefore, baseline BAFs, which are lipid normalized for comparative purposes, need to be adjusted to reflect the lipid content of aquatic organisms consumed by the target population. As discussed in the 2000 Human Health Methodology (USEPA 2000b) and the Bioaccumulation TSD (USEPA 2003), EPA recommends that where possible, lipid content of consumed aquatic species be determined on a consumption-weighted average basis.

For the purposes of deriving national AWQC, EPA has established national default consumption-weighted lipid content values of 1.9% at trophic level two, 2.6% at trophic level three, and 3.0% at trophic level four (USEPA, 2003). These national default lipid content values are based on a national survey of fish and shellfish consumption rates and information on their lipid content (see USEPA 2000b or 2003 for details of the determination of national default lipid content values). As discussed in the 2000 Human Health Methodology, EPA considers the use of national default lipid values appropriate in situations where local or regional data on lipid content and consumption rates are unavailable for the site(s) applicable to the AWQC. However, if local or regional data are available for the site(s) of interest, EPA recommends that States and Tribes use the local or regional data instead of the national default values because the type and quantity of consumed aquatic organisms and their lipid content may vary from one location to another.

6.2.3 Freely-Dissolved Fraction Applicable to AWQC

Information on the freely-dissolved fraction of the chemical expected at the site(s) applicable to the AWQC is important because the freely dissolved form of nonionic

organic chemicals is considered to represent the most bioavailable form in water and thus, the form that best predicts bioaccumulation (USEPA 2000b, 2003). Freely dissolved chemical is defined as the portion of the chemical dissolved in water, excluding the portion sorbed onto particulate and dissolved organic carbon. The freely-dissolved fraction is estimated from the octanol-water partition coefficient and the dissolved and particulate organic carbon concentrations as shown below.

$$f_{fd} = \frac{1}{\left[1 + \left(POC \bullet K_{ow}\right) + \left(DOC \bullet 0.08 \bullet K_{ow}\right)\right]} \qquad \text{[Equation 6.2.2]}$$

where:

f_{fd} = freely-dissolved fraction of chemical in water applicable to the AWQC

POC = concentration of particulate organic carbon applicable to the AWQC (kg/L)

DOC = concentration of dissolved organic carbon applicable to the AWQC (kg/L)

K_{ow} = n-octanol-water partition coefficient for the chemical

In this equation, the terms "K_{ow}" and "0.08 ℂ K_{ow}" are used to estimate the partition coefficients to POC and DOC, respectively, which have units of L/kg, the scientific basis of which is explained in USEPA (2000b and 2003). Based on national default values of 2.9 mg/L (2.9×10^{-06} kg/L) for DOC, 0.48 mg/L (4.8×10^{-07} kg/L) for POC, and 93.3 for the K_{ow} (log K_{ow} of 1.97), the freely dissolved concentration of chloroform is calculated to be 0.9999 (expressed as four significant digits for convenience), as follows:

$$f_{fd} = \frac{1}{\left[1 + \left(4.8 \times 10^{-7} \bullet 93.3\right) + \left(2.9 \text{ x } 10^{-6} \bullet 0.08 \bullet 93.3\right)\right]} = 0.9999$$

The national default values for POC and DOC used here are based on the median value of POC and DOC concentrations observed in numerous water bodies across the United States and are described further in USEPA (2000b and 2003). For the purposes of deriving national AWQC, EPA believes that the use of national default values is appropriate. In addition, EPA considers the use of national default values of POC and DOC as being appropriate in situations where local or regional data on POC and DOC are unavailable for the site(s) applicable to the AWQC. However, if local or regional data are available for the site(s) of interest, EPA recommends that States and Tribes

use the local or regional data instead of the national default values because the POC and DOC can vary on a local basis, thus affecting the freely dissolved fraction.

6.2.4 Calculation of National BAFs

The last step in deriving a national BAF for a given trophic level is to convert the final baseline BAF_R^{fd} determined in Section 6.1.2 to a BAF that reflect conditions to which the national AWQC will apply. Using Equation 6.2.1 and the inputs described above, the BAFs appropriate for calculating national AWQC are calculated as follows:

$$National\ BAF_{(TL\ n)} = [(Baseline\ BAF_R^{fd})_{TL\ n} \times (f_R)_{TL\ n} + 1] \times (f_{fd})$$

National BAF for Trophic Level Two

= [(93.3 L/kg-lipid) × (0.019 kg-lipid/kg-tissue) +1] × (0.9999)
= 2.8 L/kg-tissue

National AWQC BAF for Trophic Level Three

= [(93.3 L/kg-lipid) × (0.026 kg-lipid/kg-tissue) +1] × (0.9999)
= 3.4 L/kg-tissue

National AWQC BAF for Trophic Level Four

= [(91.7 L/kg-lipid) × (0.030 kg-lipid/kg-tissue) +1] × (0.9999)
= 3.8 L/kg-tissue[2]

[2]Use of the Baseline BAF_R^{fd} that was calculated by method 4 (i.e., from K_{ow}) results in the same National BAF as calculated by method 3 here).

7. AWQC CALCULATION

7.1 FOR AMBIENT WATERS USED FOR DRINKING WATER SOURCES

The basic equation used to calculate the AWQC that is protective of an individual who is exposed to ambient water both by ingestion of fish and by use of the ambient water as an indoor drinking water source is as follows:

$$AWQC = RfD \times RSC \times \frac{BW}{\left[IR + \sum (FI_{TLi} \times BAF_{TLi})\right]}$$

where:

RfD	=	Oral reference dose for chloroform (µg/kg-day)
RSC	=	Fraction of total exposure attributable to ambient water
BW	=	Body weight (kg)
IR	=	Ingestion rate of ambient water used for drinking (L/day)
$FI_{TL\,i}$	=	Fish intake rate for trophic level "i" (g/day)
$BAF_{TL\,i}$	=	Bioaccumulation factor for chloroform in fish of trophic level "i" (µg/g per µg/L)

Table 7-1 summarizes the input values needed to calculate the AWQC. Based on these inputs, the resulting AWQC value for this scenario is 68 µg/L.

TABLE 7-1 SUMMARY OF TOXICITY AND EXPOSURE PARAMETERS

Pathway	Variable	Units	Value	Comment	Source
All	RfD	ug/kg-d	10	Agency consensus value	See Section 4
Drinking water ingestion	RSC	--	0.20	Default "floor" value derived using decision tree approach	See Section 5
	IR	L/day	2	AWQC default value	USEPA 2000b
Fish Ingestion	RSC	--	0.2	Default "floor" value derived using decision tree approach	See Section 5
	FI (TL 2)	g/day	3.8	AWQC default value	USEPA 2000b
	FI (TL 3)	g/day	8.0	AWQC default value	USEPA 2000b
	FI (TL 4)	g/day	5.7	AWQC default value	USEPA 2000b
	BAF (TL 2)	L/kg	2.8	Calculated from available data	See Section 6
	BAF (TL 3)	L/kg	3.4	Calculated from available data	See Section 6
	BAF (TL 4)	L/kg	3.8	Calculated from available data	See Section 6

7.2 FOR AMBIENT WATERS NOT USED FOR DRINKING WATER SOURCES

The basic equation used to calculate the AWQC that is protective of an individual who is exposed to ambient water by ingestion of fish is as follows:

$$AWQC = RfD \times RSC \times \frac{BW}{\sum (FI_{TL\,i} \times BAF_{TL\,i})}$$

where:

RfD	=	Oral reference dose for chloroform (µg/kg-day)
RSC	=	Fraction of total exposure attributable to ambient water
BW	=	Body weight (kg)
$FI_{TL\,i}$	=	Fish intake rate for trophic level "i" (g/day)
$BAF_{TL\,i}$	=	Bioaccumulation factor for chloroform in fish of trophic level "i" (µg/g per µg/L)

Table 7-1 summarizes the input values needed to calculate the AWQC. Based on these inputs, the resulting AWQC value for this scenario is 2,400 µg/L.

7.3 AWQC SUMMARY

Based on the equations and input parameters described above, the AWQC values for chloroform are as follows:

AWQC Type	AWQC Value (a)
Ingestion of drinking water plus ingestion of organisms	68 µg/L
Ingestion of organisms only	2,400 µg/L

(a) AWQC values are shown to two significant figures

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8. SITE-SPECIFIC OR REGIONAL ADJUSTMENTS TO CRITERIA

Several parameters in the AWQC equation can be adjusted on a site-specific or regional basis to reflect regional or local conditions and/or specific populations of concern. These include fish consumption; incidental water consumption as related to regional/local recreational activities; BAF (including factors used to derive BAFs such as POC/DOC, percent lipid of fish consumed by target population, and species representative of given trophic levels); and the relative source contribution. States and Tribes are encouraged to make adjustments using the information and instructions provided in the 2000 Human Health Methodology (USEPA 2000b) and its supporting Technical Support Documents for Exposure Assessment (Volume II) and Bioaccumulation (Volume III) when they become available.

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