

**INTERACTION PROFILE FOR CHLOROFORM, 1,1-DICHLOROETHENE,
TRICHLOROETHYLENE, AND VINYL CHLORIDE**

**Agency for Toxic Substances and Disease Registry
U.S. Department of Health and Human Services
Public Health Service**

February 2026

PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found.

To carry out these legislative mandates, ATSDR's Office of Innovation and Analytics, Toxicology Section has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, locates available *in vivo* and *in vitro* toxicological studies evaluating mixtures, performs quantitative modeling of joint action, and develops methods for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration, or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All of the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists, in collaboration with mixtures risk assessors and laboratory scientists, have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have

thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

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PEER REVIEW

A peer review panel was assembled for this profile. The panel consisted of the following members:

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These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for the compounds evaluated in this profile.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

Chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride were chosen as the subject mixture for this profile because they frequently occur in water around hazardous waste sites. The primary routes of exposure of nearby populations to mixtures of these volatile chemicals are likely to be inhalation and oral, and the durations of concern are intermediate and chronic. ATSDR toxicological profiles are available for all four of the components of the mixture (ATSDR 2019, 2022c, 2024a, 2024b); these documents are the primary sources of information presented in the Appendices concerning the toxicokinetics, health effects, mechanisms of action, and health guidelines for these chemicals.

The purposes of this profile are to: (1) evaluate data (if available) on health hazards, and their dose-response relationships, from exposure to this four-component mixture; (2) evaluate data on the joint toxic actions of components of this mixture; and (3) make recommendations for exposure-based assessments of the potential impact of joint toxic action of the mixture on public health.

No studies were located that examined health effects in humans or animals exposed to mixtures exclusively containing chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride, and no physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models for this mixture have been developed. A component-based approach (ATSDR 2001, 2018) was applied, wherein the potential influence of individual components on the toxicity of other components in the mixture is evaluated. As joint action data are lacking for three of the six component pairs, the mechanisms of action for each component pair were also analyzed for evidence of potential joint toxic actions. The weight-of-evidence (WOE) analysis indicated that the most likely mode of joint action for the individual component pairs was competition for cytochrome P450 2E1 (CYP2E1) active sites, but only at high exposure levels where metabolic saturation may occur. Competitive inhibition of metabolism was predicted to result in less-than-additive toxicity for effects mediated through the generation of reactive metabolites (e.g., hepatic, renal, and carcinogenic effects), greater-than-additive toxicity for effects due to the toxicity of the parent compound (neurological effects of chloroform), and uncertain results for effects that may be due to both parent compound and metabolite (neurological effects of trichloroethylene) or have inadequate mechanistic data (neurological effects of vinyl chloride). Some evidence was available from acute-duration co-exposure studies in animals to support these predictions for hepatic effects.

Component-based approaches that assume endpoint-specific additive joint toxic action are recommended for exposure-based assessments of possible noncancer or cancer health hazards from inhalation exposure

to chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride, because there are no direct data available to characterize health hazards (and dose-response relationships) from the four-component mixture. The WOE analysis predicted nonadditive joint action at high exposure levels, but the mode of action (competitive inhibition of metabolism at saturating exposure levels) is not relevant to lower exposure scenarios, as would occur from exposures from water near hazardous waste sites; thus, the dose and response additivity assumptions appear to be suitable in the interest of protecting public health from noncancer and carcinogenic hazards, respectively.

The health effects or endpoints of concern for this mixture are hepatic and developmental effects (all four chemicals), renal (chloroform, 1,1-dichloroethene, trichloroethylene), neurological (chloroform, trichloroethylene, vinyl chloride), immunological (trichloroethylene, vinyl chloride), respiratory (chloroform, 1,1-dichloroethene), and cancer (chloroform, trichloroethylene, vinyl chloride). To screen this mixture for potential noncancer hazards to public health using the dose additivity approach, endpoint-specific hazard indexes are estimated using Minimal Risk Levels (MRLs) and target-organ toxicity doses (TTDs), derived in this interaction profile, for the exposure routes and durations of concern. This approach is appropriate when the hazard quotients for two or more of the mixture components equal or exceed 0.1. Endpoint-specific hazard indexes (e.g., hazard indexes for hepatic effects) for the same exposure duration (e.g., chronic) can be summed across routes (inhalation and oral) to estimate the aggregate hazard, if it is likely that the same individual or group of individuals would be exposed by both routes. The total cancer risk is estimated using the response addition approach, which involves summing the cancer risks for chloroform, trichloroethylene, and vinyl chloride. Cancer risks for the same exposure duration can be summed across routes if it is likely that the same individual or group of individuals would be exposed by both routes. If an endpoint-specific hazard index exceeds one, or the sum of the cancer risks for these chemicals equals or exceeds 1×10^{-4} , then further evaluation is needed (ATSDR 2018), using biomedical judgment and community-specific health outcome data, and considering community health concerns (ATSDR 1992). If exposures levels are very high interactions may occur (e.g., ≥ 100 -fold above the MRLs or TTDs), and their potential impact on the hazard indexes and cancer risks can be determined using the WOE predictions discussed earlier in this summary.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AKT	α -ketoglutarate transaminase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BINWOE	binary weight-of-evidence
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
CHCl ₃	chloroform
CNS	central nervous system
COCl ₂	phosgene
CYP2E1	cytochrome P450 2E1
DCVC	S-(1,2-dichlorovinyl)-L-cysteine
DCVG	S-(1,2-dichlorovinyl)glutathione
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
GD	gestation day
GGT	gamma-glutamyl transferase
GSH	glutathione
GST	glutathione S-transferase
HCFC	hydrochlorofluorocarbon
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
IARC	International Agency for Research on Cancer
IgG	immunoglobulin G
IgM	immunoglobulin M
i.p.	intraperitoneal
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
LRRK2	leucine-rich repeat kinase 2
LSE	Levels of Significant Exposure
MFO	mixed function oxidase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRL	Minimal Risk Level
NOAEL	no-observed-adverse-effect level
PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
PFC	plaque-forming cell
POD	point of departure
PVC	polyvinyl chloride
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SDH	sorbitol dehydrogenase
SPL	Substance Priority List
SRBC	Sheep red blood cell

TaClo	trichloromethyl-1,2,3,4-tetrahydro-beta-carboline
TTD	target-organ toxicity dose
VOC	volatile organic compound
WOE	weight-of-evidence

1. Introduction

The primary purpose of this *Interaction Profile for Chloroform, 1,1-Dichloroethene, Trichloroethylene, and Vinyl Chloride* is to evaluate data on the toxicology of the “whole” mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence (WOE) approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The WOE evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Office of Innovation and Analytics, Toxicology Section recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios. For more information on different approaches to evaluating mixtures and background information on chemical interactions, readers can refer to the [Framework for Assessing Health Impacts of Multiple Chemical and Other Stressors](#) (ATSDR 2018).

The chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride mixture was chosen as the subject for this interaction profile because these chemicals were among the top 10 chemicals found in water around hazardous waste sites. They are currently at the 14th, 19th, 2nd, and 18th place, respectively, as determined by number of sites the chemicals were measured in water in ATSDR's Substance Priority List (SPL) data (ATSDR 2022a). Consequently, they are also encountered in combinations. All information provided here regarding the occurrence of these chemicals is extracted from the ATSDR's SPL data (ATSDR 2022a) and data are related to completed exposure pathways (i.e., people were/are actually exposed to the chemicals; for the definition of completed exposure pathways, see ATSDR 2022b). For example, the binary combination of 1,1-dichloroethene and trichloroethylene was reported at 91 sites in water (at 108 sites in all exposure media combined). Trichloroethylene and vinyl chloride combination occurred at 97 sites, of which 72 sites had these chemicals together in water. The binary

combination of chloroform and trichloroethylene was found in water at 63 sites (total 101 sites for all media). Chloroform and 1,1-dichloroethene were found together at 46 and 34 sites for total media and water, respectively. 1,1-Dichloroethene and vinyl chloride were reported at 44 sites; 36 sites had these chemicals in water media. Finally, the binary combination of chloroform and vinyl chloride was reported at 22 sites in water (40 sites for all media). Exposure to all four chemicals together occurred at 16 sites total and at 9 sites through contaminated water. Exposure levels detected in water at contaminated sites in 2022 are shown in Table 1.

Table 1. Levels of Pollutants at Contaminated Water Sites

Chemical (number of sites)	Median (mg/L)	Minimum (mg/L)	Maximum (mg/L)
Chloroform (147)	0.036	6.00×10^{-4}	7,800
1,1-Dichloroethene (91)	0.16	2.00×10^{-4}	910
Trichloroethylene (521)	5.85	9.80×10^{-7}	3,100,000
Vinyl chloride (58)	1.14	2.00×10^{-3}	1,000

Source: ATSDR (2022a)

Previously, ATSDR developed interaction profiles for other volatile organic compounds (VOCs) found frequently in water around hazardous waste sites. These include a mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene (ATSDR 2004a) and a mixture of benzene, ethylbenzene, toluene, and xylenes (ATSDR 2004b). Before evaluating the relevance of joint toxic action data for chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride, some understanding of these chemicals and the health endpoints of concern for inhalation and oral exposure is needed. The endpoints of concern include the various critical effects that are the bases for Minimal Risk Levels (MRLs) or other health guidance values, and any other endpoints that may become significant because they are relatively sensitive shared targets of toxicity or due to interactions (ATSDR 2018).

At room temperature, chloroform is a colorless, volatile liquid with a pleasant, nonirritating odor and slightly sweet taste. Chloroform may be found in the environment as a result of industrial production and use (mainly in the manufacture of the refrigerant, hydrochlorofluorocarbon-22 [HCFC-22]) or from generation of chloroform during water disinfection with chlorine. Following inhalation or oral exposure to chloroform, the most sensitive effects are on the liver and respiratory system; effects on the kidney, nervous system, and developing organism have also been reported. High-dose chloroform has been used as an anesthetic, but it is no longer used for that purpose. Many of chloroform's effects are believed to be the result of metabolism to active products that react with target tissues. The Department of Health and

Human Services' (HHS) *Fifteenth Report on Carcinogens* (NTP 2021a) states that chloroform is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. The U.S. Environmental Protection Agency (EPA) has classified chloroform as *likely to be carcinogenic to humans* according to the 1999 cancer guidelines (EPA 1999) based on sufficient evidence of carcinogenicity in animals at sufficiently high exposure conditions that produce sustained cytotoxicity and regenerative hyperplasia (EPA 2001). The International Agency for Research on Cancer (IARC) classifies chloroform as *possibly carcinogenic to humans* (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC 1999). More information on chloroform is provided in Appendix A and ATSDR (2024a).

At room temperature, 1,1-dichloroethene is a colorless, highly volatile liquid with a mild, sweet smell. The primary source of 1,1-dichloroethene in the environment is industrial production and use (to make polyvinylidene chloride copolymers for plastics, flexible wraps, and flame-retardant coatings).

1,1-Dichloroethene's primary effects following exposure are on the respiratory tract (inhalation), liver, kidney, and developing organism. Many of 1,1-dichloroethene's effects are believed to be the result of metabolism to active products that react with target tissues. The HHS *Fifteenth Report on Carcinogens* (NTP 2021b) does not list 1,1-dichloroethene. EPA (2002) has classified 1,1-dichloroethene (listed as 1,1-dichloroethylene) as having *suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential* via the inhalation route according to the 1999 cancer guidelines (EPA 1999) based on animal data (data are inadequate to assess its carcinogenic potential via the oral route). IARC (2019) notes that 1,1-dichloroethene (listed as vinylidene chloride) is *possibly carcinogenic to humans* (Group 2B) based on sufficient evidence in experimental animals. More information on 1,1-dichloroethene is provided in Appendix B and ATSDR (2022c).

At room temperature, trichloroethylene is a colorless, volatile liquid with a somewhat sweet odor. It is used primarily as a solvent and may be found in numerous industrial applications as well as in paint removers, adhesives, and spot removers. Following inhalation or oral exposure, trichloroethylene is metabolized to active metabolites including trichloroacetic acid and trichloroethanol. The primary effects of trichloroethylene and its metabolites are neurological (altered visual-motor coordination, drowsiness), and additional effects by the metabolites including hepatic, renal, immunological, and developmental are also reported. The HHS *Fifteenth Report on Carcinogens* (NTP 2021c) states that trichloroethylene is *reasonably anticipated to be a human carcinogen* based on sufficient evidence from human studies. EPA (2011) classified trichloroethylene as *carcinogenic to humans* according to the 2005 cancer guidelines (EPA 2005) based on sufficient epidemiological evidence. IARC (2014) lists trichloroethylene as

Group 1 (*carcinogenic to humans*) based on sufficient evidence in humans and experimental animals. More information on trichloroethylene is provided in Appendix C and ATSDR (2019).

Vinyl chloride is a colorless gas at room temperature, which at very high concentrations, has a mild, sweet odor. It is commonly used industrially, mainly in the production of polyvinyl chloride (PVC) polymers. The majority of its effects are believed to result from metabolism to active intermediates, which then react with target tissues. The most sensitive effects of inhalation or oral exposure to low levels of vinyl chloride have been reported in the liver; immunological, neurological, and developmental effects also have been reported following inhalation exposures. The HHS *Fifteenth Report on Carcinogens* (NTP 2021d) reports that vinyl chloride is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans. EPA (2000) classified vinyl chloride as a *known/likely human carcinogen* according to the proposed 1996 cancer guidelines (EPA 1996) based on epidemiological data. IARC (2012) lists vinyl chloride as *carcinogenic to humans (Group 1)* based on sufficient evidence of carcinogenicity in humans and animals. More information on vinyl chloride is provided in Appendix D and ATSDR (2024b).

ATSDR toxicological profiles are available for all four of the chemicals that make up the mixture (ATSDR 2019, 2022c, 2024a, 2024b); these documents are the primary source of information presented in the appendices concerning the toxicokinetics, health effects, mechanisms of action, and health guidelines for these chemicals. The various critical effects that are the bases for the MRLs, as well as other relatively sensitive effects, are summarized in Table 2. All four chemicals are known to have effects on the liver and developing organism. The nervous and renal systems are common targets of three of the chemicals, and the immunological and respiratory systems are common targets of two of the chemicals. Carcinogenicity is an endpoint of concern for three of the chemicals. No pertinent studies of the toxicity or interactions of, or of PBPK models for, the complete mixture or any of the tertiary submixtures were located. Limited joint toxic action data are available for three of the individual component binary mixtures, and metabolic data and PBPK models are available for three of the binary mixtures.

Table 2. Potential Sensitive Health Effects of Concern for Intermediate- and Chronic-Duration Inhalation and Oral Exposure to the Mixture, Chloroform, 1,1-Dichloroethene, Trichloroethylene, and Vinyl Chloride^{a,b}

Endpoint	Chloroform	1,1-Dichloroethene	Trichloroethylene	Vinyl chloride
Hepatic	X	X	X	X
Renal	X	X	X	
Immunological			X	X ^c
Respiratory	X^c	X^c		
Neurological	X		X	X
Developmental	X	X	X	X ^c
Cancer	X		X	X

^aSee Appendices A, B, C, and D.

^bThe basis for the intermediate- and chronic-duration MRLs are bolded; other sensitive effects are listed in regular typeface.

^cInhalation only.

2. Joint Toxic Action Data for the Mixture of Concern and Binary Mixtures of Components

2.1 Mixture of Concern

Toxicological data or PBPK models were not available for the complete mixture of concern.

2.2 Binary Mixtures of Components

Toxicological data or PBPK models were not available for any of the three-component submixtures. Only limited toxicological data were available for the binary mixtures. However, joint metabolic data were available for chloroform and trichloroethylene, for trichloroethylene and 1,1-dichloroethene, and for trichloroethylene and vinyl chloride. Rodent PBPK models have been developed for these three binary mixtures.

In the following sections on the binary mixtures, the studies that focus on toxic endpoints are discussed first, followed by studies of pharmacokinetic effects and relevant PBPK models. At the end of each binary mixture section, the experimental results that may be used to support conclusions regarding joint toxic action are summarized in tables. For each listed endpoint and study, the tables present a conclusion regarding the direction of interaction for the influence of each chemical on the toxicity of the other. These conclusions include additive (dose addition, response addition, or no apparent influence), greater than additive (synergism or potentiation), less than additive (antagonism, inhibition, or masking), or indeterminate (ambiguous, conflicting, or no data).

2.2.1 Chloroform and 1,1-Dichloroethene

No *in vivo* or *in vitro* studies were located regarding joint toxic actions of chloroform and 1,1-dichloroethene. No PBPK models specific for co-exposure to chloroform and 1,1-dichloroethene were located. For both compounds, however, bioactivation by cytochrome P450 2E1 (CYP2E1) is required for toxicity for the majority of effects, so a possible interaction can be hypothesized along that pathway (ATSDR 2022c, 2024a). At very high co-exposure levels, when the enzyme is saturated, the toxicities of chloroform and 1,1-dichloroethene could be expected to decrease for the majority of sensitive endpoints due to mutual inhibition of each other's metabolism. However, since the neurotoxic effects of chloroform are believed to result from the parent compound, rather than a metabolite, it is anticipated that

the neurotoxic effects from chloroform will be more prominent when metabolism is saturated. Mechanistic details for chloroform are provided in Appendix A and for 1,1-dichloroethene are provided in Appendix B.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.2 Chloroform and Trichloroethylene

One study examined the joint effects of acute-duration intraperitoneal (i.p.) administration of chloroform and trichloroethylene on liver endpoints in rats (Anand et al. 2005a). Groups of three male Sprague-Dawley rats were injected contralaterally with low (74+250 mg/kg), moderate (185+500 mg/kg), and high (370+1,250 mg/kg) (chloroform+trichloroethylene) dose combinations. Controls were injected with the corn oil vehicle. Results for the binary mixture, presented primarily for the moderate- and high-dose groups, were compared with those obtained with each chemical alone and with what would be predicted from response addition. Neither the single chemicals nor the binary mixtures resulted in mortality. Plasma alanine aminotransferase (ALT), measured at 24-hour intervals from 24 to 96 hours after dosing and evaluated as the area under the curve (AUC), was significantly lower in the mixture groups than predicted by the sum of measured responses to each chemical alone, indicating a less-than-additive joint toxic action. In the discussion section of the study publication, the study authors stated that no frank histopathological changes were seen in the livers of the rats exposed to the binary mixture, and that this finding was similar to their previous results with chloroform alone, in which increased plasma ALT occurred without histopathological changes, perhaps due to slight cell membrane damage. They further stated that trichloroethylene alone cause midzonal liver injuries in their previous studies. No mention of histopathological examination was included in the results sections of the Anand et al. (2005a) publication, so these statements cannot be evaluated.

Anand et al. (2005a) also investigated the effects of joint i.p. administration on the disposition of chloroform and trichloroethylene in the rats exposed as described above. Trichloroethylene had no significant effect on blood or liver concentrations of chloroform except for an apparent decrease in blood concentrations of chloroform during the first 0.5 hour after dosing in the mixture group as compared with the chloroform alone group at the high-dose level. Trichloroethylene concentrations were significantly decreased in the mixture group as compared with trichloroethylene-alone group in blood at 1–6 hours after dosing and in liver at 1 hour after dosing. Concentrations of trichloroethylene in urine at 6 hours after dosing (but not at 12 and 24 hours after dosing) were significantly higher in the mixture groups than

in the corresponding trichloroethylene-alone groups. A major route for excretion of unmetabolized trichloroethylene, however, is through the expired air, which was not monitored. The blood and liver concentrations of trichloroacetic acid (trichloroethylene metabolite) were lower in the mixture groups than in the corresponding trichloroethylene alone groups, and the AUCs (6–48 hours) of urine concentrations of trichloroacetic acid and trichloroethanol (another trichloroethylene metabolite) also were decreased in the mixture groups as compared with the trichloroethylene-alone groups. Some of the effects are consistent with inhibition of trichloroethylene metabolism by chloroform, but the decreased blood concentrations of trichloroethylene suggest that other mechanisms also may be significant. These results suggest a greater influence of chloroform on the disposition and metabolism of trichloroethylene than *vice versa* at the doses tested and for the i.p. route. Their applicability to other routes of exposure is uncertain.

Other identified studies assessing the potential interactions between chloroform and trichloroethylene evaluated mixtures containing additional chemicals, precluding the usefulness to draw specific conclusions regarding interactions between the binary mixture of chloroform and trichloroethylene. Anand et al. (2005b) investigated the joint effects of acute-duration i.p. administrations of a tertiary mixture of chloroform, trichloroethylene, and allyl alcohol to rats. This study demonstrated an antagonistic effect of the three-chemical mixture versus the single chemicals on liver effects and trichloroethylene disposition and metabolism, but response-additive results from the two-chemical mixture of trichloroethylene and allyl alcohol suggested that the antagonism in the three-chemical mixture was between chloroform and trichloroethylene. This suggestion was further investigated by Anand et al. (2005a, see previous paragraph). Another study in rats examined the hepatotoxic effects of drinking water exposure to mixtures of chemicals for up to 6 months that included chloroform and trichloroethylene (Constan et al. 1995), but only a seven-chemical organic and inorganic mixture and a four-chemical organic submixture were evaluated, so information on the possible joint actions of chloroform and trichloroethylene could not be determined.

A PBPK model specific for inhalation co-exposure to chloroform and trichloroethylene is described below. For both compounds, bioactivation by CYP2E1 is required for induction of some toxic effects (see Appendices A and C), so a possible interaction can be hypothesized through mutual inhibition of CYP2E1 metabolism resulting in less-than-additive toxicity. This interaction would be expected only at relatively high exposure levels where the enzyme is saturated. Since the neurotoxic effects of chloroform are believed to result from the parent compound, rather than a metabolite, it is anticipated that the

neurotoxic effects of chloroform will be more prominent during high levels of co-exposure with trichloroethylene. For trichloroethylene, both the parent compound and a metabolite, trichloroethanol, are neurotoxic. Limited evidence suggests that trichloroethanol may be more potent than trichloroethylene in altering electrical excitability of the motor cerebral cortex, threshold current intensity of electrical skin stimulation, and electroencephalogram and electrocardiograph results in guinea pigs administered i.p. doses (Mikisková and Mikiska 1966), and that blood levels of trichloroethanol correlate better with visual evoked potentials and electroretinographic measurements than blood levels of trichloroethylene following inhalation exposure to the parent compound (Blain et al. 1992, 1994). Therefore, the impact of chloroform inhibition of trichloroethylene metabolism is uncertain.

A joint PBPK model for chloroform and trichloroethylene in the rat was developed for inhalation exposure by Isaacs et al. (2004). The model consists of five non-metabolizing compartments (alveolar space, lung blood, fat, slowly perfused tissue, and rapidly perfused viscera) and two metabolizing compartments (liver for both chemicals and kidney for chloroform). Kinetic constants and inhibitory parameters were estimated from gas uptake experiments using 70–80-day-old male F344 rats exposed for 6 hours in a closed chamber. The gas uptake experiments for single-chemical exposures included separate exposures of three rats per concentration at initial chamber concentrations of 100, 500, 1,000, or 3,000 ppm, with concentrations monitored at 10-minute intervals. The mixture exposures were conducted with one chemical as substrate and the other as inhibitor, and *vice versa*, at the following initial chamber concentrations: 1,000 ppm substrate and 1,000 ppm inhibitor (one rat); 500 ppm substrate and 500 ppm inhibitor (two rats); 500 ppm substrate and 10 ppm inhibitor (three rats); and 500 ppm substrate and 2,000 ppm inhibitor (three rats). Chamber substrate concentrations were measured at 10-minute intervals. A comparison of model simulations with the gas uptake data indicated that a purely competitive model for metabolic interaction was the most appropriate fit to the data for either chemical treated as substrate. The study did not attempt to identify a threshold region for metabolic interaction.

Only one study of the joint toxic action of chloroform and trichloroethylene was located (Anand et al. 2005a). This study was concerned only with hepatic effects and was conducted by i.p. injection in rats. The data are summarized in Table 3 and evaluation of plasma ALT responses determined a less-than-additive interaction at the tested doses. This result is consistent with the PBPK model predictions of competitive inhibition of metabolism, which would be expected to result in less-than-expected toxicities associated with reactive metabolites.

Table 3. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Chloroform and Trichloroethylene

Route, duration	Endpoint	Results for response addition			Conclusions	Reference
		Greater than additive	Additive/no apparent influence	Less than additive		
Intraperitoneal, acute	Hepatic (plasma ALT)			185–370 mg/kg CHCl ₃ + 500–1,250 mg/kg TCE (rats)	Less-than-additive for hepatic effects of both chemicals	Anand et al. 2005a

ALT = alanine aminotransferase; CHCl₃ = chloroform; TCE = trichloroethylene

2.2.3 Chloroform and Vinyl Chloride

No *in vivo* or *in vitro* studies were located regarding joint toxic actions of chloroform and vinyl chloride. No PBPK models specific for co-exposure to chloroform and vinyl chloride were located. For both compounds, however, bioactivation by CYP2E1 is required for induction of some toxic effects, so a possible interaction can be hypothesized through mutual inhibition of CYP2E1 metabolism (ATSDR 2024a, 2024b). Thus, at very high co-exposure levels, when the enzyme is saturated, the toxicities of chloroform and vinyl chloride could be expected to decrease for the majority of sensitive endpoints, due to mutual inhibition of each other's metabolism. Since the neurotoxic effects of chloroform are believed to result from the parent compound, rather than a metabolite, it is anticipated that the neurotoxic effects will be more prominent when metabolism is saturated. Mechanistic details for chloroform are provided in Appendix A and for vinyl chloride are provided in Appendix D.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.4 1,1-Dichloroethene and Trichloroethylene

Andersen et al. (1987) reported that inhalation co-exposure of groups of four male F344 rats to 500 ppm trichloroethylene and a range of concentrations of 1,1-dichloroethene (~100–1,800 ppm) for a single 6-hour exposure resulted in a protective effect on hepatotoxicity, as assessed by serum aspartate aminotransferase (AST) measurement, compared to 1,1-dichloroethene alone, with higher levels of 1,1-dichloroethene in the mixture required to elicit the same serum AST increases. The study authors reported that similar changes were noted in serum ALT levels, but data were not provided in the study.

The level of metabolism of 1,1-dichloroethene estimated by a PBPK model (see below) correlated strongly with the changes in hepatic enzyme levels seen in the study, indicating that the protective effect was likely due to inhibition of metabolism of 1,1-dichloroethene. A protective effect of trichloroethylene on 1,1-dichloroethene lethality also was apparent.

Similarly, El-Masri et al. (1996a) found that inhalation exposure of F344 rats (number and sex not specified) to 500 or 1,000 ppm trichloroethylene for a single 3.5–4.5-hour exposure inhibited the hepatotoxicity (as indicated by serum AST) of simultaneous exposure to 1,000 ppm 1,1-dichloroethene, but that 50 and 100 ppm trichloroethylene did not have a significant inhibitory effect. Despite the study reporting limitations (e.g., number of animals/sex), findings reported by the study authors are supported by the large magnitude of observed response, reported statistics, and similar findings reported by Andersen et al. (1987).

A joint PBPK model for 1,1-dichloroethene and trichloroethylene in the rat was developed by Andersen et al. (1987). The model consists of five compartments (gas exchange, slowly perfused, rapidly perfused, fat, and liver) with metabolism assumed to occur only in the liver compartment. Andersen and Dennison (2004) later described the model as having four compartments (liver, fat, slowly perfused tissues, and rapidly perfused tissues) and gas exchange with the exposure atmosphere. A comparison of model simulations gas uptake data from co-exposure of 1,1-dichloroethene and trichloroethylene in male F344 rats indicated that a purely competitive model for metabolic interaction at cytochrome P450 was the most appropriate fit to the data. A later report describing additional simulations and experimental data (El-Masri et al. 1996b) further confirmed a competitive interaction between 1,1-dichloroethene and trichloroethylene, and the model predicted that no interaction would be observed below 100 ppm of either chemical. The model was further refined to predict hepatic glutathione (GSH) content (which is depleted by CYP2E1-generated metabolites of 1,1-dichloroethene) following exposure to the two compounds, and again, predicted no interaction below 100 ppm and competitive inhibition of CYP2E1 metabolism at higher concentrations (El-Masri et al. 1996a). Thus, the threshold for competitive inhibition in the rat was estimated to be >100 ppm of each chemical.

Two studies of the joint toxicity of 1,1-dichloroethene and trichloroethylene were located, and those studies evaluated only hepatic endpoints and only to a limited degree. These studies are summarized in Table 4 and indicate a less-than additive effect of trichloroethylene on the hepatotoxicity of 1,1-dichloroethene, which is consistent with the PBPK model predictions of competitive inhibition of

metabolism above 100 ppm, which would be expected to result in less-than-additive toxicities associated with reactive metabolites.

Table 4. Summary of Available Data on the Joint Effects of Simultaneous Exposure to 1,1-Dichloroethene and Trichloroethylene

Route, duration	Endpoint	Results for response addition			Conclusions	Reference
		Greater than additive	Additive/no apparent influence	Less than additive		
Inhalation, acute	Hepatic (serum AST and ALT)			500 ppm TCE + 100–1,800 ppm DCE (rats)	Less-than-additive effect of TCE on DCE hepatic effects	Andersen et al. 1987
	Hepatic (serum AST)		50–100 ppm TCE + 1,000 ppm DCE (rats)	500–1,000 ppm TCE + 1,000 ppm DCE (rats)	Less-than-additive effect of TCE on DCE hepatic effects at ≥ 500 ppm	El-Masri et al. 1996a

ALT = alanine aminotransferase; AST = aspartate aminotransferase; DCE = 1,1-dichloroethene; TCE = trichloroethylene

2.2.5 1,1-Dichloroethene and Vinyl Chloride

Jaeger et al. (1975) conducted a series of inhalation experiments in which groups of ≈ 5 male Holtzman rats were exposed to high levels of 1,1-dichloroethene and vinyl chloride, either together or in succession. 1,1-Dichloroethene exposure alone, at ≈ 200 ppm for 4 hours followed by a 6-hour observation period, resulted in an increase in serum alanine α -ketoglutarate transaminase (AKT) (an alternate name for ALT) over unexposed controls, as well as the development of hepatic midzonal necrosis. Exposure to very high ($\approx 46,000$ ppm) levels of vinyl chloride for 4 hours did not result in changes in serum AKT or in hepatic injury. According to the study authors, vinyl chloride is not known to produce an immediate hepatotoxic response. Simultaneous exposure of fasted male rats to 1,000 ppm of vinyl chloride and 200 ppm of 1,1-dichloroethene (a hepatotoxic concentration) resulted in no changes in serum AKT activity or hepatic histopathology, indicating a protective effect of vinyl chloride on 1,1-dichloroethene's acute hepatotoxicity. Only when exposure to vinyl chloride was reduced to 201 ppm in another group of rats, exposures to $\approx 2,000$ ppm of 1,1-dichloroethene resulted in severe liver damage and increased AKT activity, which required the animals to be sacrificed *in extremis* before the end of the evaluation period; co-exposure to 12,000 ppm of vinyl chloride completely negated the increased AKT activity. The above results for exposure to each chemical separately and for simultaneous exposures to both chemicals were

obtained with fasted rats, which were more sensitive to 1,1-dichloroethene hepatotoxicity due to depletion of GSH by fasting. GSH conjugation detoxifies the reactive metabolites of 1,1-dichloroethene. Pretreatment of fed rats with 10,600 ppm vinyl chloride for 5 hours (which also results in depletion of GSH), followed by 2,000 ppm 1,1-dichloroethene for 4 hours, resulted in increased serum AKT and sorbitol dehydrogenase (SDH) levels, whereas exposure of fed rats to 2,000 ppm 1,1-dichloroethene alone did not increase these indices of liver damage.

No PBPK models specific for co-exposure to 1,1-dichloroethene and vinyl chloride were located. For both compounds, however, bioactivation by CYP2E1 is required for toxicity (see Appendices B and D), so a possible interaction can be hypothesized along that pathway. At very high co-exposure levels, when the enzyme is saturated, the joint toxicities of 1,1-dichloroethene and vinyl chloride on most endpoints could be expected to decrease due to competitive inhibition of each other's metabolism. Limited data are available to support this possible interaction pathway. Jaeger et al. (1975) evaluated the effects of relatively high-concentration inhalation co-exposure on hepatic endpoints in fasted rats and reported less-than-additive joint toxicity consistent with competitive inhibition of metabolism, but data on low-concentration exposures or on non-hepatic endpoints are not available, and fasted rats are unusually sensitive to 1,1-dichloroethene hepatotoxicity. Results of a sequential exposure experiment were consistent with vinyl chloride depletion of GSH resulting in greater-than-additive hepatotoxicity from 1,1-dichloroethene in fed rats (Jaeger et al. 1975). These joint toxic action studies of 1,1-dichloroethene and vinyl chloride are summarized in Table 5.

Table 5. Summary of Available Data on the Joint Effects of Simultaneous and Sequential Exposure to 1,1-Dichloroethene and Vinyl Chloride

Route, duration	Endpoint	Results for response addition		Conclusions	Reference	
		Greater than additive	Additive/no apparent influence			Less than additive
Simultaneous exposure						
Inhalation, acute	Hepatic (serum ALT, liver lesions)			Up to 12,000 ppm VC + 2,000 ppm DCE (rats)	Less-than-additive effect of VC on DCE hepatic effects	Jaeger et al. 1975
Sequential exposure						
Inhalation, acute	Hepatic (serum ALT, SDH)	12,600 ppm VC, then 2,000 ppm DCE (rats)			Greater-than-additive effect of VC on DCE hepatic effects	Jaeger et al. 1975

ALT = alanine aminotransferase; DCE = 1,1-dichloroethene; SDH = sorbitol dehydrogenase; VC = vinyl chloride

2.2.6 Trichloroethylene and Vinyl Chloride

Barton et al. (1995) reported that acute, high-dose inhalation co-exposure of three to six male Sprague-Dawley rats to trichloroethylene and vinyl chloride ($\leq 5,000$ ppm for ≤ 6 hours) resulted in decreased depletion of hepatic nonprotein sulfhydryl groups, compared to exposure to vinyl chloride alone. Controls were sham exposed. Depletion of nonprotein sulfhydryls (e.g., GSH) occurs from conjugation with reactive vinyl chloride metabolites. A dose-response effect was observed in rats exposed to 1,000 and 5,000 ppm of vinyl chloride alone, but not ≤ 600 ppm of vinyl chloride alone. At 5,000 ppm vinyl chloride, the depletion of GSH was 44% of the control value. In contrast, trichloroethylene alone at $\leq 5,000$ ppm did not appreciably deplete GSH. While this study provides insight into metabolic interactions between trichloroethylene and vinyl chloride, no endpoints of toxicity were evaluated. Results from exposure to the mixture are presented in Table 6.

Table 6. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Trichloroethylene and Vinyl Chloride

Route, duration	Endpoint	Results on response additivity			Conclusions	Reference								
		Greater than additive	Additive/no apparent influence	Less than additive										
Simultaneous exposure														
Inhalation, acute	Hepatic/metabolic (depletion of non-protein sulfhydryls)			<table border="1"> <thead> <tr> <th>VC:TCE</th> <th>%</th> </tr> </thead> <tbody> <tr> <td>5,000:600</td> <td>35</td> </tr> <tr> <td>5,000:5,000</td> <td>20</td> </tr> <tr> <td>1,000:1,000</td> <td>22</td> </tr> </tbody> </table>	VC:TCE	%	5,000:600	35	5,000:5,000	20	1,000:1,000	22	Less-than-additive effect of TCE on VC (metabolic competitive inhibition)	Barton et al. 1995
VC:TCE	%													
5,000:600	35													
5,000:5,000	20													
1,000:1,000	22													

TCE = trichloroethylene; VC = vinyl chloride; VC:TCE concentrations in ppm; % = percentage of depleted non-protein sulfhydryls

Barton et al. (1995) developed a PBPK model for inhalation co-exposure to trichloroethylene and vinyl chloride in the rat, based on a previously published model for trichloroethylene and 1,1-dichloroethene (Andersen et al. 1987). The model consisted of five compartments (gas exchange, slowly perfused, rapidly perfused, fat, and liver), with metabolic components modeled in the liver compartment. The model simulations were compared to acute-duration inhalation co-exposure data from gas uptake experiments in groups of three male Sprague-Dawley rats assuming competitive, noncompetitive, and uncompetitive inhibition models for shared metabolism by CYP2E1. At concentrations <30 ppm for each chemical, there was no noticeable effect of either compound on the uptake or metabolism of the other. Above that concentration, the PBPK model indicated that the chemicals displayed behavior characteristic of competitive inhibition of the P450 enzyme, rather than uncompetitive or noncompetitive inhibition. Trichloroethylene was found to be a more effective inhibitor of vinyl chloride metabolism than *vice versa*, which the study authors attributed to the higher blood:air partition coefficient of trichloroethylene that results in a higher blood concentration at the same external exposure concentration. The PBPK predictions are consistent with the effects on hepatic nonprotein sulfhydryl levels seen following co-exposure (Barton et al. 1995) and suggest a less-than-additive joint action at high exposure concentrations for toxicity mediated through metabolites. For environmental scenarios, Barton et al. (1995) concluded that the pharmacokinetics of the compounds would be independent (competitive inhibition would not occur); therefore, additive joint actions would occur.

Tohon et al. (2019) developed a PBPK model for multi-route exposures to trichloroethylene and vinyl chloride. The exposure routes evaluated included ingestion of contaminated drinking water as well as

inhalation and dermal exposures from bathing in contaminated water for 30 minutes. Two concentrations were evaluated: the “low” exposure water concentrations were set to the EPA’s Drinking Water Health Advisories (EPA 2018) of 0.2 and 3 mg/L for trichloroethylene and vinyl chloride, respectively, while the “high” exposure water concentrations were 10 times the “low” exposure values (i.e., 2 mg/L for trichloroethylene and 30 mg/L for vinyl chloride). The corresponding trichloroethylene and vinyl chloride air concentrations were 0.2 and 12.4 ppb, respectively, for the “low” exposure scenario and 2.2 and 124.4 ppb, respectively, for the “high” exposure scenario. The model consisted of seven compartments (pulmonary exchange, liver, highly perfused tissues, fat, skin, kidney, and rest of the body), with metabolism of trichloroethylene and vinyl chloride by CYP2E1 occurring in the liver and metabolism of trichloroethylene by glutathione S-transferases (GSTs) occurring in the liver and kidneys. To evaluate age-related toxicokinetic differences, Tohon et al. (2019) included age-specific physiological parameters, including cardiac output, liver volume, and CYP2E1 concentration, for infants (2–6 months old), toddlers (7–24 months old), children (2–10 years old), teenagers (11–17 years old), and adults (18–64 years old) in the model. For the “low” and “high” exposure levels, models were run for each chemical alone and for the co-exposure scenario.

The modeling results indicated no appreciable inhibition of trichloroethylene CYP2E1 metabolism by vinyl chloride or vice versa under the “low” exposure condition for any age group. In contrast, inhibition of CYP2E1-mediated metabolism of both compounds by the other occurred under the “high” exposure condition as demonstrated by decreased formation of CYP2E1-dependent metabolites (decreases of 7.3–22% for vinyl chloride and 38.5–45.5% for trichloroethylene) and a corresponding increase in the parent compound AUC (increases of 8–22.6% for vinyl chloride and 27–42% for trichloroethylene) relative to the values generated for the single chemical exposures. The metabolic inhibition was evident for all age groups, and the total doses (parent compound AUCs) decreased with age for the single and co-exposure conditions. For trichloroethylene, the decreased formation of CYP2E1-mediated metabolites during the co-exposure corresponded to increases in GST-mediated metabolites in the liver+kidneys (26.2–44.6%) and kidneys alone (24–41.8%), although the masses of CYP2E1-mediated metabolites produced were still orders of magnitude greater than the masses of GST-mediated metabolites formed. Tohon et al. (2019) defined a variability index (VI) as the ratio of the 95th percentile amounts of metabolites formed (via CYP2E1 or GSTs) or the parent compound AUC for populations younger than adults (infants, toddlers, children, and teenagers) to the corresponding median adult value. All VIs were <2.5 for all age comparisons for both chemicals alone or as mixtures, with higher values identified for infants and toddlers and a decreasing trend as the populations increased with age.

Joint PBPK models have been described (Barton et al. 1995; Tohon et al. 2019) that predict: (1) no interaction between trichloroethylene and vinyl chloride at inhalation exposure levels <30 ppm of each and (2) a less-than-additive interaction on metabolism at air concentrations >30 ppm and drinking water concentrations greater than EPA's Drinking Water Health Advisories of 0.2 and 3 mg/L for trichloroethylene and vinyl chloride, respectively. Thus, the threshold for metabolic interaction was predicted to be >30 ppm for inhalation exposures and greater than the EPA's Drinking Water Health Advisories for drinking water exposures. The competitive inhibition of each other's metabolism at the higher exposure levels would be expected to result in less-than-additive joint action for toxicities mediated through reactive metabolites.

2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health

The exposure routes for the chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride mixture in water near hazardous waste sites are anticipated to be inhalation, owing to the volatility of the chemicals, and oral. Anticipated exposure durations of concern are primarily intermediate to chronic. No epidemiological or toxicological studies of the complete mixture or any of the three-component submixtures are available. No PBPK models are available for the complete mixture; however, for three of the two-component submixtures, animal PBPK models have been developed. Some information and studies are available for binary mixtures of the components, but they are not adequate to support a quantitative assessment of interactions. Therefore, the WOE approach is appropriate (ATSDR 2001, 2018) to predict the potential impact of interactions. This approach involves determining, for each binary mixture, the WOE for the influence of one component on the toxicity of the other, and *vice versa*.

The binary weight-of-evidence (BINWOE) classification scheme is summarized in Table 7. This table gives a general idea of the approach, which rates confidence in the predicted direction of interaction according to the quality of the data. The direction of interaction is predicted from the available mechanistic and toxicological data, and the quality of the data, as it pertains to prediction of direction of interaction, is classified by the main data quality factors for *mechanistic understanding* and *toxicological significance*. If concerns regarding the applicability of the data are not completely addressed under the main data quality factors, they can be addressed by the use of the *modifiers*. More detailed guidance is given in ATSDR guidance documents (ATSDR 2001, 2018). Rationales for the BINWOE determinations are presented in Tables 8–19 at the end of this section. The BINWOE determinations are presented for the binary mixtures in the same order as these mixtures were considered in Section 2.2.

Table 7. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

Classification	
Direction of Interaction	
=	Additive
>	Greater than additive
<	Less than additive
?	Indeterminate
Quality of the Data	
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.
A.	The toxicological significance of the interaction has been directly demonstrated.
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.
C.	The toxicological significance of the interaction is unclear.
1.	Anticipated exposure duration and sequence.
2.	Different exposure duration or sequence.
a.	<i>In vivo</i> data
b.	<i>In vitro</i> data
i.	Anticipated route of exposure
ii.	Different route of exposure

Evidence of varying quality and quantity is available supporting projections of joint toxic action for the six unique binary pairs of the four chemicals of concern:

- chloroform and 1,1-dichloroethene
- chloroform and trichloroethylene
- chloroform and vinyl chloride
- 1,1-dichloroethene and trichloroethylene
- 1,1-dichloroethene and vinyl chloride
- trichloroethylene and vinyl chloride

While data on the joint toxic actions of three of the individual component pairs are not available, mechanistic and/or joint exposure metabolic data suggest that under conditions of metabolic saturation at higher exposures, less-than-additive interactions (due to competitive inhibition of CYP2E1 metabolism) may occur for each of the component pairs; the exception to this are the neurological effects elicited by chloroform, which are believed to be due to the parent compound and would therefore be more prominent

under conditions of co-exposure at metabolic saturation. However, it appears unlikely that metabolic saturation will be a significant factor at the exposure levels typically seen from water near hazardous waste sites.

Table 8. Effect of Chloroform on 1,1-Dichloroethene

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for respiratory effects
BINWOE: <IIBb for developmental effects

Direction of Interaction – Because both chloroform and 1,1-dichloroethene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. This would be expected to result in less-than-additive toxicity at very high doses of chloroform and 1,1-dichloroethene.

Mechanistic Understanding – Many of the effects of chloroform are due to the formation of reactive intermediates, including phosgene, following metabolism by cytochrome P450 enzymes, particularly CYP2E1 (Appendix A). 1,1-Dichloroethene is similarly metabolized by CYP2E1 to reactive intermediates (Appendix B), and therefore, may compete for the enzyme at high exposure levels. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related binary mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels for chloroform and 1,1-dichloroethene below which no interaction would be expected have not been measured or estimated. Since the direct mechanism of the interaction has not been characterized but can be inferred from the individual mechanisms of action of the compounds, and from related binary mixtures, a rating of “II” was assigned for mechanistic understanding.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were located in which pretreatment with chloroform prior to 1,1-dichloroethene exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of “b” was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 9. Effect of 1,1-Dichloroethene on Chloroform

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for respiratory effects
BINWOE: >IIBb for neurological effects
BINWOE: <IIBb for developmental effects
BINWOE: <IIBb for carcinogenic effects

Direction of Interaction – Because both 1,1-dichloroethene and chloroform are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. This would be expected to result in less-than-additive toxicity at very high doses of 1,1-dichloroethene and chloroform. Because the neurological effects of chloroform are not believed to result from reactive metabolism, but from the parent compound, they are anticipated to be increased under conditions of metabolic saturation.

Mechanistic Understanding – 1,1-Dichloroethene is metabolized by cytochrome P450 enzymes, primarily CYP2E1, to reactive metabolites, which are believed to cause its toxic effects (Appendix B). Many of the effects of chloroform are similarly due to the formation of reactive intermediates, including phosgene, following metabolism by CYP2E1 (Appendix A). At high exposure levels, it is possible that the two compounds could compete for active enzyme. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals to their respective toxic metabolites. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related binary mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels for 1,1-dichloroethene and chloroform below which no interaction would be expected have not yet been measured or estimated. The mechanism of chloroform-induced neurological effects is thought to involve the parent compound (Appendix A). As such, under co-exposure conditions where metabolism is saturated, chloroform's neurological effects would be expected to be more pronounced. Since the direct mechanism of the interaction has not been characterized but can be inferred from the individual mechanisms of action of the compounds, and from related binary mixtures, a rating of "II" was assigned for mechanistic understanding.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were located in which pretreatment with 1,1-dichloroethene prior to chloroform exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene, and trichloroethylene), a rating of "B" was assigned.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of "b" was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 10. Effect of Chloroform on Trichloroethylene

BINWOE: <IAii for hepatic effects
BINWOE: <IBii for renal effects
BINWOE: <IBii for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IBii for developmental effects
BINWOE: <IBii for carcinogenic effects

Direction of Interaction – Because both chloroform and trichloroethylene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies, and a less-than-additive joint toxic action of these chemicals on liver effects has been reported in an acute-duration, high-dose intraperitoneal study. Because the neurological effects of trichloroethylene may result both from the parent compound and from metabolites (e.g., trichloroethanol), no estimate of the direction of possible interactions can be made for that endpoint.

Mechanistic Understanding – Many of the effects of chloroform are due to the formation of reactive intermediates, including phosgene, following metabolism by cytochrome P450 enzymes, particularly CYP2E1 (Appendix A). Trichloroethylene is similarly metabolized by CYP2E1 to reactive intermediates (Appendix C) and could therefore be hypothesized to compete for the enzyme at high exposure levels. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals. Analysis of the behavior of this interaction using a joint PBPK model developed for the two chemicals (Isaacs et al. 2004), developed with inhalation data in rats, indicated that the interaction was best modeled when competitive inhibition of the two compounds for the active site was assumed. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related two-chemical mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels for chloroform and trichloroethylene below which no interaction would be expected have not yet been measured or modeled. Also, the neurological effects of trichloroethylene may be due to both the parent compound and metabolites (Appendix C); therefore, it is not possible to predict the extent of the interactions between the two chemicals for this endpoint based on available mechanistic data. Since a direct demonstration of the mechanism by which the interactions could occur exists, a rating of “I” was assigned for mechanistic understanding for all endpoints other than neurological.

Toxicological Significance – A single study in rats, using simultaneous acute-duration, intraperitoneal administration, demonstrated less-than-additive liver toxicity from chloroform and trichloroethylene in combination than from either chemical by itself (Anand et al. 2005a). These results are consistent with the mechanistic understanding. Since the toxicological significance of the interaction was demonstrated in a single study, a rating of “A” may be appropriate for hepatic effects. A rating of “B” is appropriate for the other toxicities thought to be mediated through the same mechanism, but which were not investigated in this study. A rating of “?” was assigned for neurological effects because mechanistic data indicate that both the parent compound and metabolites (e.g., trichloroethanol) may contribute to observed effects; therefore, the impact of metabolic saturation due to co-exposure with chloroform cannot be predicted in the absence of experimental data.

Modifying Factors – Because of concerns regarding the applicability of intraperitoneal data to inhalation or oral exposure, a modifying factor of “ii” is applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 11. Effect of Trichloroethylene on Chloroform

BINWOE: <IAii for hepatic effects
BINWOE: <IBii for renal effects
BINWOE: <IBii for respiratory effects
BINWOE: >IBii for neurological effects
BINWOE: <IBii for developmental effects
BINWOE: <IBii for carcinogenic effects

Direction of Interaction – Because both trichloroethylene and chloroform are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies, and a less-than-additive joint toxic action of these chemicals on liver effects has been reported in an acute-duration, intraperitoneal study. Because the neurological effects of chloroform are not believed to result from reactive metabolism, but from the parent compound, they are anticipated to be increased under conditions of metabolic saturation.

Mechanistic Understanding – Trichloroethylene is metabolized by cytochrome P450 enzymes, particularly CYP2E1, to active metabolites, which are believed to cause its toxic effects (Appendix C). Many of the effects of chloroform are due to the formation of reactive intermediates, including phosgene, following metabolism by CYP2E1 (Appendix A). At high exposure levels, it is possible that the two compounds could compete for active enzymes. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals to their respective toxic metabolites. Analysis of the behavior of this interaction using a joint PBPK model developed for the two chemicals (Isaacs et al. 2004), developed with inhalation data in rats, indicated that the interaction was best modeled when competitive inhibition of the two compounds for the active site was assumed. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related two-chemical mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride); the exposure level below which no interaction would be expected has not yet been measured or modeled. The mechanism of chloroform-induced neurological effects is thought to involve the parent compound (Appendix A). As such, under conditions where metabolism is saturated, the neurological effects would be expected to be more pronounced. Since a direct demonstration of the mechanism by which the interactions could occur exists, a rating of “I” was assigned for mechanistic understanding.

Toxicological Significance – A single study in rats, using simultaneous acute-duration, intraperitoneal administration, demonstrated less-than-additive liver toxicity from chloroform and trichloroethylene in combination than from either chemical by itself (Anand et al. 2005a). These results are consistent with the mechanistic understanding. Since the toxicological significance of the interaction was demonstrated in a single study, a rating of “A” may be appropriate for hepatic effects. A rating of “B” is appropriate for the other toxicities thought to be mediated through the same mechanism, but which were not investigated in this study.

Modifying Factors – Because of concerns regarding the applicability of intraperitoneal data to inhalation or oral exposure, a modifying factor of “ii” is applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 12. Effect of Chloroform on Vinyl Chloride

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IIBb for developmental effects
BINWOE: <IIBb for carcinogenic effects

Direction of Interaction – Because both vinyl chloride and chloroform are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. This would be expected to result in less-than-additive toxicity at very high doses of vinyl chloride and chloroform.

Mechanistic Understanding – Many of the effects of chloroform are due to the generation of reactive intermediates, including phosgene, following metabolism by cytochrome P450 enzymes, particularly CYP2E1 (Appendix A). Vinyl chloride is similarly metabolized by CYP2E1 to reactive products (Appendix D) and could therefore be hypothesized to compete for the enzyme at high exposure levels. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related binary mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels of chloroform and vinyl chloride below which no interaction would be expected have not yet been measured or estimated. Since the direct mechanism of the interaction has not been characterized but can be inferred from the individual mechanisms of action of the compounds, and from related binary mixtures, a rating of “II” was assigned for mechanistic understanding all endpoints other than neurological. Since the mechanisms of neurotoxicity have not been characterized, it is not possible to predict the extent of the interactions between the two chemicals for this endpoint based on available mechanistic data.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were located in which pretreatment with chloroform prior to vinyl chloride exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned for all endpoints other than neurological. A rating of “?” was assigned for neurological effects because available mechanistic data are insufficient to determine if neurotoxicity is attributable to the parent compound, a metabolite, or both; therefore, the impact of metabolic saturation due to co-exposure with chloroform cannot be predicted in the absence of experimental data.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of “b” was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 13. Effect of Vinyl Chloride on Chloroform

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for respiratory effects
BINWOE: >IIBb for neurological effects
BINWOE: <IIBb for developmental effects
BINWOE: <IIBb for carcinogenic effects

Direction of Interaction – Because both vinyl chloride and chloroform are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. This would be expected to result in less-than-additive toxicity at very high doses of vinyl chloride and chloroform. Because the neurological effects of chloroform are not believed to result from reactive metabolism, but from the parent compound, they are anticipated to be increased under conditions of metabolic saturation.

Mechanistic Understanding – Many of the effects of chloroform are due to the generation of reactive intermediates, including phosgene, following metabolism by cytochrome P450 enzymes, particularly CYP2E1 (Appendix A). Vinyl chloride is similarly metabolized by CYP2E1 to reactive products, and therefore, could be hypothesized to compete for the enzyme at high exposure levels (Appendix D). In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related binary mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels of vinyl chloride and chloroform below which no interaction would be expected have not yet been measured or estimated. Also, the mechanism of chloroform-induced neurological effects is thought to involve the parent compound (Appendix A). As such, under conditions where metabolism is saturated the neurological effects would be expected to be more prevalent. Since the direct mechanism of the interaction has not been characterized but can be inferred from the individual mechanisms of action of the compounds, and from related binary mixtures, a rating of “II” was assigned for mechanistic understanding.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were located in which pretreatment with vinyl chloride prior to chloroform exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of “b” was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 14. Effect of 1,1-Dichloroethene on Trichloroethylene

BINWOE: <IB for hepatic effects
BINWOE: <IB for renal effects
BINWOE: <IB for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IB for developmental effects
BINWOE: <IB for carcinogenic effects

Direction of Interaction – Because both 1,1-dichloroethene and trichloroethylene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies. This would be expected to result in less-than-additive toxicity at very high doses of 1,1-dichloroethene and trichloroethylene. Because the neurological effects of trichloroethylene may result both from the parent compound and from the metabolite trichloroethanol, no estimate of the direction of possible interactions can be made for that endpoint.

Mechanistic Understanding – Many of the effects of trichloroethylene are believed to be the result of metabolism by CYP2E1 to reactive metabolites (Appendix C). Inhalation studies in rats have shown that at high doses, 1,1-dichloroethene can compete with trichloroethylene for CYP2E1 active sites, resulting in a less-than-additive metabolic interaction. Analysis of the behavior of this interaction using a joint PBPK model developed for the two chemicals (Andersen et al. 1987; El-Masri et al. 1996a, 1996b) indicated that the interaction was best modeled when competitive inhibition of the two compounds for the active site was assumed. Later applications of the model, when compared with experimental results, further confirmed competitive inhibition for CYP2E1, and demonstrated that at concentrations below 100 ppm, no evidence of any interaction between the two compounds could be demonstrated. This would be consistent with competitive inhibition, which would require enzyme saturation in order to result in differences in effects, and would therefore exhibit a threshold response. Because the neurological effects of trichloroethylene may be due to both the parent compound and to the metabolite trichloroethanol (Appendix C), it is not possible to predict the extent of the interactions between the two chemicals for this endpoint based on available mechanistic data. Since a direct demonstration of the mechanism by which the interactions could occur exists, a rating of “I” for mechanistic understanding was assigned for all endpoints except neurological.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. Although Andersen et al. (1987) and El-Masri et al. (1996a) reported acute, co-exposure inhalation studies of trichloroethylene and 1,1-dichloroethene in rats, no toxic effects of trichloroethylene were observed in the studies. No studies were found in which pretreatment with 1,1-dichloroethene prior to trichloroethylene exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for a related binary mixture (chloroform and trichloroethylene), a rating of “B” was assigned.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 15. Effect of Trichloroethylene on 1,1-Dichloroethene

BINWOE: <IA for hepatic effects
BINWOE: <IB for renal effects
BINWOE: <IB for respiratory effects
BINWOE: <IB for developmental effects

Direction of Interaction – Because both trichloroethylene and 1,1-dichloroethene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies, and a less-than-additive influence of co-exposure to trichloroethylene on the hepatotoxicity of 1,1-dichloroethene has been reported in two acute-duration inhalation studies in rats.

Mechanistic Understanding – The effects of 1,1-dichloroethene are believed to be the result of metabolism by CYP2E1 to reactive metabolites, which then react with target tissues to cause toxicity (Appendix B). Inhalation studies in rats have shown that at high doses, trichloroethylene can compete with 1,1-dichloroethene for CYP2E1 active sites, resulting in a less-than-additive metabolic interaction. Analysis of the behavior of this interaction using a joint PBPK model developed for the two chemicals (Andersen et al. 1987; El-Masri et al. 1996a, 1996b) indicated that the interaction was best modeled when competitive inhibition of the two compounds for the active site was assumed. Later applications of the model, when compared with experimental results, further confirmed competitive inhibition for CYP2E1 and demonstrated that at concentrations below 100 ppm for both compounds, no evidence of any interaction between the two compounds could be demonstrated. This would be consistent with competitive inhibition, which would require enzyme saturation in order to result in differences in effects and would therefore exhibit a threshold response. Since a direct demonstration of the mechanism by which the interactions could occur exists, a rating of “I” for mechanistic understanding was assigned.

Toxicological Significance – Andersen et al. (1987) reported that acute-duration inhalation co-exposure to 500 ppm trichloroethylene and a range of concentrations of 1,1-dichloroethene (~100–1,800 ppm) resulted in a protective effect on hepatotoxicity compared to 1,1-dichloroethene alone. El-Masri et al. (1996a) further reported that acute-duration inhalation co-exposure to 500 or 1,000 ppm trichloroethylene inhibited the hepatotoxicity of simultaneous exposure to 1,000 ppm 1,1-dichloroethene, but that 50 and 100 ppm trichloroethylene did not have a significant inhibitory effect. No studies were found in which pretreatment with trichloroethylene prior to 1,1-dichloroethene exposure was examined. Since the toxicological significance of the interaction was demonstrated in two studies, and is consistent with the mechanistic data, a rating of “A” is appropriate for hepatic effects. A rating of “B” is appropriate for the other toxicities thought to be mediated through the same mechanism, but which were not investigated in this study.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 16. Effect of 1,1-Dichloroethene on Vinyl Chloride

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IIBb for developmental effects
BINWOE: <IIBb for carcinogenic effects

Direction of Interaction – Because both 1,1-dichloroethene and vinyl chloride are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. This would be expected to result in less-than-additive toxicity at very high doses of 1,1-dichloroethene and vinyl chloride.

Mechanistic Understanding – 1,1-Dichloroethene is metabolized by cytochrome P450 enzymes, particularly CYP2E1, to active metabolites, which are believed to cause its toxic effects (Appendix B). Similarly, many of the effects of vinyl chloride are believed to be due to the formation of reactive products following metabolism by CYP2E1 (Appendix D). At high exposure levels, it is possible that the two compounds could compete for active enzymes. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals to their respective toxic metabolites. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related two-chemical mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels of 1,1-dichloroethene and vinyl chloride below which no interaction would be expected have not yet been measured or estimated. Since the direct mechanism of the interaction has not been directly characterized but can be inferred from the individual mechanisms of action of the compounds, a rating of “II” was assigned for mechanistic understanding for mechanistic understanding all endpoints other than neurological. Since the mechanisms of neurotoxicity have not been characterized, it is not possible to predict the extent of the interactions between the two chemicals for this endpoint based on available mechanistic data.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. Jaeger et al. (1975) reported an acute, co-exposure inhalation study of vinyl chloride and 1,1-dichloroethene in rats, but no toxic effects of vinyl chloride were reported in the study. No studies were located in which pretreatment with 1,1-dichloroethene exposure prior to vinyl chloride was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned for all endpoints other than neurological. A rating of “?” was assigned for neurological effects because available mechanistic data are insufficient to determine if neurotoxicity is attributable to the parent compound, a metabolite, or both; therefore, the impact of metabolic saturation due to co-exposure with 1,1-dichloroethene cannot be predicted in the absence of experimental data.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of “b” was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 17. Effect of Vinyl Chloride on 1,1-Dichloroethene

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for respiratory effects
BINWOE: <IIBb for developmental effects

Direction of Interaction – Because both vinyl chloride and 1,1-dichloroethene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive effect of co-exposure to vinyl chloride on the hepatotoxicity of 1,1-dichloroethene was seen in an acute, high-exposure study in fasted rats.

Mechanistic Understanding – 1,1-Dichloroethene is metabolized by CYP2E1 to reactive intermediates that are believed to be the cause of its toxicity (Appendix B). Similarly, vinyl chloride is metabolized by CYP2E1 to reactive products, which result in its toxic effects (Appendix D) and could therefore be hypothesized to compete for the enzyme at high exposure levels. In such a case, the toxicity of both compounds would be expected to be reduced, as insufficient amounts of the enzyme would be available to fully metabolize both chemicals. This was demonstrated for acute-duration co-exposure by Jaeger et al. (1975) who reported that acute, high-dose co-exposure of rats to vinyl chloride, which is less acutely hepatotoxic than 1,1-dichloroethene, reduced or eliminated the hepatotoxicity of 1,1-dichloroethene exposure. At low doses, however, it is likely that no detectable interaction would be noticed, similar to what has been demonstrated for related two-chemical mixtures that have been evaluated (1,1-dichloroethene and trichloroethylene, trichloroethylene and vinyl chloride); the exposure level below which no interaction would be expected has not yet been measured or estimated. Since the direct mechanism of the interaction has not been characterized but can be inferred from the individual mechanisms of action of the compounds, a rating of “II” was assigned for mechanistic understanding.

Toxicological Significance – Jaeger et al. (1975) reported that acute, inhalation co-exposure to high concentrations of 1,1-dichloroethene and vinyl chloride in rats resulted in reduction of the toxicity seen with 1,1-dichloroethene alone; this study used fasted rats, which are depleted in GSH. Studies of longer durations or more environmentally relevant concentrations were not located. In fed rats, pre-exposure to an extremely high concentration of vinyl chloride (which also depletes GSH), resulted in an increased hepatotoxicity of subsequent exposure to 1,1-dichloroethene (Jaeger et al. 1975). The simultaneous exposure study is considered more relevant in terms of sequence. Although the toxicological significance has been demonstrated for this chemical pair and for similar binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene) some uncertainty exists due to the differential fasted/fed experimental designs and outcomes of the simultaneous and sequential studies, and therefore a rating of “B” was assigned.

Modifying Factors – Because the mechanistic data are based primarily on *in vitro* studies, a modifier of “b” was applied.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 18. Effect of Trichloroethylene on Vinyl Chloride

BINWOE: <IB for hepatic effects
BINWOE: <IB for renal effects
BINWOE: <IB for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IB for developmental effects
BINWOE: <IB for carcinogenic effects

Direction of Interaction – Because both trichloroethylene and vinyl chloride are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies. This would be expected to result in less-than-additive toxicity at high doses of trichloroethylene and vinyl chloride.

Mechanistic Understanding – Many of the effects of vinyl chloride are believed to be the result of metabolism by CYP2E1 to a reactive metabolite, which then can bind to tissue molecules to produce cellular damage (Appendix D). Trichloroethylene is also metabolized primarily by CYP2E1 to form reactive products (Appendix C), so competition for the active enzyme at high doses is possible. A five-compartment joint rat PBPK model for vinyl chloride and trichloroethylene has been developed (Barton et al. 1995) and compared with high-dose inhalation data. A comparison of model simulations with experimental co-exposure data indicated that a competitive model of metabolism, where the two chemicals are assumed to independently compete for the active site of the enzyme, best fit the available metabolic data. It was also noted that at concentrations below 30 ppm, there was no noticeable effect of either compound on the uptake or metabolism of the other. Competitive metabolism is also supported by a seven-compartment joint human PBPK model evaluating oral, inhalation, and dermal exposures for multiple age groups exposed to trichloroethylene and vinyl chloride water concentrations of 2 and 30 mg/L, respectively (Tohon et al. 2019). Since a direct demonstration of the mechanism by which the interactions could occur exists and has been replicated in human modeling, a rating of “I” for mechanistic understanding was assigned for mechanistic understanding all endpoints other than neurological. Since the mechanisms of neurotoxicity have not been characterized, it is not possible to predict the extent of the interactions between the two chemicals for this endpoint based on available mechanistic data.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were found in which pretreatment with trichloroethylene prior to vinyl chloride exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned for all endpoints other than neurological. A rating of “?” was assigned for neurological effects because available mechanistic data are insufficient to determine if neurotoxicity is attributable to the parent compound, a metabolite, or both; therefore, the impact of metabolic saturation due to co-exposure with trichloroethylene cannot be predicted in the absence of experimental data.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 19. Effect of Vinyl Chloride on Trichloroethylene

BINWOE: <IB for hepatic effects
BINWOE: <IB for renal effects
BINWOE: <IB for immunological effects
BINWOE: ? for neurological effects
BINWOE: <IB for developmental effects
BINWOE: <IB for carcinogenic effects

Direction of Interaction – Because both vinyl chloride and trichloroethylene are metabolized to reactive metabolites by the same enzyme, it is anticipated that once metabolism is saturated, the effects of each will be lessened due to a limitation on the rate of production of new metabolites. A less-than-additive interaction at the level of metabolism has been verified in high-dose animal studies. This would be expected to result in less-than-additive toxicity at high doses of trichloroethylene and vinyl chloride. Because the neurological effects of trichloroethylene may be due to both the parent compound and the metabolite trichloroethanol, the possible effects of vinyl chloride on trichloroethylene-induced neurological effects cannot be determined.

Mechanistic Understanding – Many of the effects of trichloroethylene are believed to be the result of metabolism by CYP2E1 to a reactive metabolite, which then can bind to tissue molecules to produce cellular damage (Appendix C). Vinyl chloride is also metabolized primarily by CYP2E1 to form reactive products (Appendix D), so competition for the active enzyme at high doses is possible. A five-compartment joint rat PBPK model for vinyl chloride and trichloroethylene has been developed (Barton et al. 1995) and compared with high-dose inhalation data. A comparison of model simulations with experimental co-exposure data indicated that a competitive model of metabolism, where the two chemicals are assumed to independently compete for the active site of the enzyme, best fit the available metabolic data. It was also noted that at concentrations below 30 ppm, there was no noticeable effect of either compound on the uptake or metabolism of the other. The seven-compartment joint human PBPK model developed by Tohon et al. (2019) used to evaluate multi-route exposures for multiple age groups exposed to trichloroethylene and vinyl chloride water concentrations of 2 and 30 mg/L, respectively, also support competitive metabolism of vinyl chloride and trichloroethylene by CYP2E1. Since a direct demonstration of the mechanism by which the interactions could occur exists and has been corroborated in human modeling, a rating of “I” for mechanistic understanding was assigned. Because the neurological effects of trichloroethylene may be due to both the parent compound and to the metabolite trichloroethanol (Appendix C), it is not known how competitive interaction for CYP2E1 would affect this endpoint.

Toxicological Significance – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were found in which pretreatment with vinyl chloride prior to trichloroethylene exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred and has been demonstrated for related binary mixtures (chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene), a rating of “B” was assigned.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

2.4 Recommendations for Data Needs

Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the four-component mixture, or for three-component submixtures, are available. Similarly, PBPK models describing the behavior of the four-component mixture, or the three-component submixtures, are not available. In the absence of direct interaction data, a component-based approach was utilized. While PBPK models are available for three of the two-component submixtures, the models have been developed for rodents only and have not been expanded to allow for predictions in humans. For all the components of the mixture, metabolism by CYP2E1 appears to be an important step in the toxicity of the component. Data on thresholds from available PBPK models in rats are summarized in Table 20. Development of PBPK models for humans is needed. Obtaining human measurements of these chemicals in exhaled air and urine would enhance the credibility of the predictions.

Table 20. PBPK Models Predictions of Interaction Thresholds

Binary mixtures	Thresholds in rats	References
Chloroform and trichloroethylene	None established	Isaacs et al. 2004
1,1-Dichloroethene and trichloroethylene	>100 ppm for each chemical	El-Masri et al. 1996b
Vinyl chloride and trichloroethylene	>30 ppm for each chemical	Barton et al. 1995

Data on the toxic action of the binary submixtures following co-exposure or pre-exposure scenarios are needed for three of the binary submixtures; limited data were available for the chloroform and trichloroethylene, 1,1-dichloroethene and trichloroethylene, and trichloroethylene and vinyl chloride binary submixtures. Obtaining measurement of the chemicals (and/or metabolites) in exhaled air and urine of exposed humans would be helpful in enhancing the credibility of derived WOE_s.

For the individual components, inhalation MRLs are available for all exposure durations for chloroform, for the intermediate and chronic durations for 1,1-dichloroethene and trichloroethylene, and for acute and intermediate durations for vinyl chloride. Oral MRLs are available for all exposure durations for chloroform, for the chronic duration for 1,1-dichloroethene and vinyl chloride, and for the acute and intermediate durations for trichloroethylene. The inhalation MRLs for chloroform and the intermediate-duration inhalation MRL for 1,1-dichloroethene identify respiratory effects as the critical effects. The oral MRLs for chloroform, the chronic-duration oral MRL for 1,1-dichloroethene, and the intermediate-duration inhalation and chronic-duration oral MRLs for vinyl chloride identify hepatic effects as the critical effects. The chronic-duration inhalation and oral MRLs for trichloroethylene are based on

immunotoxicity and developmental toxicity. The acute-duration inhalation MRL for vinyl chloride is based on developmental toxicity. The available MRLs are summarized in Table .

Table 21. Minimal Risk Levels (MRLs) for the Chemicals of Concern				
	Chloroform	1,1-Dichloroethene	Trichloroethylene	Vinyl chloride
Inhalation				
Acute	0.001 ppm (respiratory effects)	None	None	0.5 ppm (developmental effects)
Intermediate	0.0008 ppm (respiratory effects)	0.001 ppm (respiratory effects)	0.0004 ppm (developmental and immunological effects)	0.02 ppm (hepatic effects)
Chronic	0.0004 ppm (respiratory effects)	0.001 ppm (respiratory effects)	0.0004 ppm (developmental and immunological effects)	None
Oral				
Acute	0.3 mg/kg/day (hepatic effects)	None	None	None
Intermediate	0.1 mg/kg/day (hepatic effects)	None	0.0005 mg/kg/day (developmental and immunological effects)	None
Chronic	0.02 mg/kg/day (hepatic effects)	0.05 mg/kg/day (hepatic effects)	0.0005 mg/kg/day (developmental and immunological effects)	0.003 mg/kg/day (hepatic effects)

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

As discussed above, the mixture of chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride was chosen as the subject for this interaction profile because these chemicals frequently occur in water around hazardous waste sites. The exposure scenarios of greatest concern for the complete mixture are likely to be inhalation (owing to the volatility of the individual components) and oral exposure for intermediate and chronic durations. Each of the four chemicals of concern cause toxic effects in two or more target organs. Three of the chemicals have been identified as likely or known carcinogenic agents (chloroform, trichloroethylene, and vinyl chloride). Separate approaches are recommended for noncancer and carcinogenic effects. All recommendations discussed below are intended to be used in consultation with the [*Framework for Assessing Health Impacts of Multiple Chemical and Other Stressors*](#) (ATSDR 2018).

Because suitable data, joint action models, and PBPK models are lacking for the complete mixture, the recommended approach for the exposure-based assessment of noncancer joint toxic action of this mixture for noncancer endpoints is to use the hazard index method with the target-organ toxicity dose (TTD) modification and qualitative WOE method to assess the potential consequences of dose-additive and interactive joint action of the components of the mixture. These methods are to be applied only under circumstances involving significant exposure to the mixture (i.e., only if hazard quotients for two or more of the compounds are ≥ 0.1) (Figure 1 of ATSDR 2018). Hazard quotients are the ratios of exposure estimates to noncancer health guideline values, such as MRLs. If only one or if none of the compounds have a hazard quotient that is ≥ 0.1 , then no further assessment of the joint toxic action is needed because dose additivity and/or interactions are unlikely to result in significant noncancer health hazard. As discussed in ATSDR (2018), the exposure-based screening for potential health hazard is used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard. While the available scientific evidence suggests less-than-additive interactions among these components for most binary mixtures (with available data), interactions are only expected at very high exposure levels that saturate metabolism. Therefore, in the interest of public health protection, the recommended approach for most exposure-based assessments is assumed dose additivity.

The TTD modification of the hazard index requires the estimation of route, duration, and endpoint-specific (target-organ-specific) hazard indexes for the endpoints of concern for a particular mixture. The

noncancer endpoints of concern for a mixture are the critical effects of the individual components, and toxicity targets in common that may become significant due to additivity or interactions. For this mixture, the endpoints of concern are hepatic, renal, immunological, respiratory, neurological, and developmental effects. Therefore, these endpoints are candidates for TTD development for the components of this mixture. The TTDs were derived as described in the appendices to this document, using the methods recommended by ATSDR (2001, 2018). BINWOEs have been developed for these endpoints also, as presented in Section 2.3, and summarized later in Section 3. The derived TTD values for intermediate-duration inhalation exposure are listed in Table 22, which also lists the intermediate-duration inhalation MRLs for each chemical.

Table 22. MRLs and TTDs for Intermediate-Duration Inhalation Exposure to Chemicals of Concern^a

Endpoint	Chemical			
	Chloroform (ppm)	1,1-Dichloroethene (ppm)	Trichloroethylene (ppm)	Vinyl chloride (ppm)
Hepatic	0.03	0.04	1	0.02 (intermediate-duration MRL)
Renal	0.03	0.01	1	Not applicable
Immunological	Not applicable	Not applicable	0.0004 (intermediate-duration MRL)	0.02
Respiratory	0.0008 (intermediate-duration MRL)	0.001 (intermediate-duration MRL)	Not applicable	Not applicable
Neurological	0.006	Not applicable	0.04	0.02
Developmental	0.03	0.05	0.0004 (intermediate-duration MRL)	0.5 (acute-duration MRL)

^aSee Appendices A, B, C, and D.

With the exception of chloroform, adequate chronic-duration inhalation data are not available for most of the endpoints of concern for the chemicals that make up the mixture. However, as described in the appendices to this document, the pharmacokinetics of the compounds are similar, with the compounds in general being rapidly absorbed, metabolized by the same enzymes, and eliminated reasonably rapidly from the body. As such, chloroform was used as the model chemical for consideration of chronic-duration TTDs, and chronic-duration TTD values for chloroform were derived in Appendix A. The chronic-duration inhalation MRL for chloroform is 0.0004 ppm and the intermediate-duration inhalation

MRL is 0.0008 ppm, with both being based on similar physiological effects (i.e., respiratory tract changes). As this difference is approximately half an order of magnitude ($10^{0.5}$) and because of the pharmacokinetic similarities and similar mode of action among the chemicals of the mixture, it is recommended that only for this mixture and the inhalation route, when chronic-duration data are lacking, the intermediate-duration inhalation TTDs and MRLs for 1,1-dichloroethene, trichloroethylene, and vinyl chloride be adjusted using a modifying factor of 3 ($10^{0.5}$) when being considered in a chronic-duration exposure scenario. The chronic-duration inhalation TTD values are presented in Table , along with the chronic-duration inhalation MRL for chloroform.

Table 23. MRLs and TTDs for Chronic-Duration Inhalation Exposure to Chemicals of Concern^a

Endpoint	Chemical			
	Chloroform (ppm)	1,1-Dichloroethene (ppm)	Trichloroethylene (ppm)	Vinyl chloride (ppm)
Hepatic	0.03	0.02	0.3	0.007
Renal	0.03	0.004	0.7	Not applicable
Immunological	Not applicable	Not applicable	0.0004 (chronic-duration MRL)	0.02
Respiratory	0.0004 (chronic-duration MRL)	0.001 (chronic-duration MRL)	Not applicable	Not applicable
Neurological	0.006	Not applicable	0.01	0.02
Developmental	0.03	0.02	0.0004 (chronic-duration MRL)	0.2

^aSee Appendices A, B, C, and D.

TTDs also were derived for oral exposure as described in the appendices to this document, using the methods recommended by ATSDR (2001, 2018), and are listed, along with MRLs, in Table 24 for intermediate-duration exposure and Table 25 for chronic-duration exposure.

Table 24. MRLs and TTDs for Intermediate-Duration Oral Exposure to Chemicals of Concern^a

Endpoint	Chemical			
	Chloroform (mg/kg/day)	1,1-Dichloroethene (mg/kg/day)	Trichloroethylene (mg/kg/day)	Vinyl chloride (mg/kg/day)
Hepatic	0.1 (intermediate- duration MRL)	0.3	0.7	0.003
Renal	0.1	0.3	2	Not applicable
Immunological	Not applicable	Not applicable	0.0005 (intermediate- duration MRL)	Insufficient data
Neurological	0.1	Not applicable	0.0005	0.04
Developmental	0.1	0.3	0.0005 (intermediate- duration MRL)	Insufficient data

^aSee Appendices A, B, C, and D.

Table 25. MRLs and TTDs for Chronic-Duration Oral Exposure to Chemicals of Concern^a

Endpoint	Chemical			
	Chloroform (mg/kg/day)	1,1-Dichloroethene (mg/kg/day)	Trichloroethylene (mg/kg/day)	Vinyl chloride (mg/kg/day)
Hepatic	0.02 (chronic-duration MRL)	0.05 (chronic-duration MRL)	0.7	0.003 (chronic-duration MRL)
Renal	0.1	0.05	0.4	Not applicable
Immunological	Not applicable	Not applicable	0.0005 (chronic- duration MRL)	Insufficient data
Neurological	0.02	Not applicable	0.0005	0.04
Developmental	0.02	0.05	0.0005 (chronic- duration MRL)	Insufficient data

^aSee Appendices A, B, C, and D.

A hazard index is calculated for each effect, route, and exposure duration of concern, using the MRLs and TTDs listed in Tables 22, 23, 24, and 25, or newer values as they become available. The hazard index is unitless so the exposure and guidance values must be in the same units (e.g., ppm). This process is shown, using intermediate-duration inhalation hepatic effects as an example, in the following equation:

$$HI_{HEPATIC} = \frac{E_{CHCl_3}}{MRL_{CHCl_3}} + \frac{E_{DCE}}{MRL_{DCE}} + \frac{E_{TCE}}{TTD_{TCE,HEPATIC}} + \frac{E_{VC}}{MRL_{VC}}$$

where $HI_{HEPATIC}$ is the intermediate-duration inhalation hazard index for hepatic toxicity, E_{CHCl_3} is the intermediate-duration inhalation exposure to chloroform (in ppm), MRL_{CHCl_3} is the intermediate-duration inhalation MRL for chloroform (based on hepatic effects, in ppm), E_{DCE} is the intermediate-duration inhalation exposure to 1,1-dichloroethene (in ppm), MRL_{DCE} is the intermediate-duration inhalation MRL for 1,1-dichloroethene (based on hepatic effects, in ppm), E_{TCE} is the intermediate-duration inhalation exposure to tetrachloroethylene (in ppm), $TTD_{TCE,HEPATIC}$ is the intermediate-duration inhalation TTD for hepatic effects of trichloroethylene (in ppm), E_{VC} is the intermediate-duration inhalation exposure to vinyl chloride (in ppm), and MRL_{VC} the intermediate-duration inhalation MRL for vinyl chloride (based on hepatic effects, in ppm). The process can be then repeated for each endpoint of concern for intermediate-duration inhalation exposure, using the appropriate exposure concentrations and TTDs/MRLs, resulting in endpoint-specific hazard indices for each effect of concern for the mixture. The same process can be carried out for chronic-duration inhalation exposure, using chronic-duration exposure concentrations and chronic-duration inhalation TTDs and MRLs, and for intermediate- and chronic-duration oral exposure, for which the exposures are estimated as oral intakes in mg/kg/day, consistent with the units of the intermediate- and chronic-duration oral MRLs and TTDs. Components for which data are not available, and therefore no TTD can be derived, or which do not affect the endpoint are not included in the endpoint-specific hazard index calculations.

If the hazard index for effects on a noncancer endpoint of concern for any duration and route is >1 , it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2018). The impact of interactions from the WOE analysis also is considered. For this particular mixture, the available data and pharmacokinetic models on the component pairs support less-than-additive interactions for the individual pairs for most endpoints attributed to reactive metabolites, as shown in Table 26; for neurological effects of chloroform, the available mechanisms suggest greater-than-additive interactions, and for the neurological effects of trichloroethylene and vinyl chloride, the direction of interaction is indeterminate. However, since the mechanism behind those interactions is likely to only occur at very high (e.g., ≥ 100 -fold times the corresponding MRL or TTD values) exposure levels, it is not likely to be a significant contributor at exposure levels resulting from water near hazardous waste sites.

Table 26. Matrix of BINWOE Determinations for Simultaneous Exposure to High Levels of Chemicals of Concern^a

		ON THE TOXICITY OF			
		Chloroform	1,1-Dichloroethene	Trichloroethylene	Vinyl chloride
EFFECT OF	Chloroform		<IIBb h,rn,rs,d	<IAii h <IBii rn,i,d,c ? n	<IIBb h,i,d,c ? n
	1,1-Dichloroethene	<IIBb h,rn,rs,d,c >IIBb n		<IB h,rn,i,d,c ? n	<IIBb h,r,i,d,c ? n
	Trichloroethylene	<IAii h <IBii rn,rs,d,c >IBii n	<IA h <IB rn,rs,d		<IB h,r,i,d,c ? n
	Vinyl chloride	<IIBb h,rn,rs,d,c >IIBb n	<IIBb h,rn,rs,d	<IB h,rn,i,d,c ? n	

^aBINWOE scheme was explained in Table 7 (ATSDR 2001, 2018). Some BINWOEs are based on results from high-level, acute-duration exposure studies (see details in Tables 8–19). Additivity is likely at low-level exposures; dose additivity is assumed for noncancer effects and response additivity is assumed for carcinogenic effects.

c = carcinogenic; d = developmental; h = hepatic; i = immunological; n = neurological; rn = renal; rs = respiratory; ? = not known

The default cancer risk assessment approach for a multi-component mixture for which no data on the carcinogenicity of the mixture are available and no PBPK models have been validated involves summing the component cancer risks, which is a good approximation of response addition for low component risks. The carcinogenic risk for each component is calculated by multiplying lifetime inhalation and oral exposure estimates for each component by the appropriate EPA cancer inhalation unit risk (an estimate of cancer risk per unit of exposure) and oral slope factor, respectively. If only one or if none of the component risks is $\geq 1 \times 10^{-6}$, then no further assessment of joint toxic action is needed due to the low likelihood that additivity and/or interactions would result in a significantly enhanced health hazard. The nonadditive interactions between the components are not likely to be significant factors at the generally low exposure levels encountered from contaminated water near hazardous waste sites. Cancer risk can be estimated for chloroform, trichloroethylene, and vinyl chloride.

If this screening procedure indicates preliminary evidence of a mixture's health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2018). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).

Where exposure of the same individual or group of individuals to this mixture may occur for the same duration by both inhalation and oral routes, it is appropriate to sum corresponding endpoint-specific hazard indices and total cancer risks across routes to estimate aggregate hazard or risk. If an endpoint-specific aggregate hazard index is >1 , or the aggregate cancer risks for these chemicals is $\geq 1 \times 10^{-4}$, then further evaluation is needed (ATSDR 2018), using biomedical judgment and community-specific health outcome data, and considering community health concerns (ATSDR 1992).

In the event of high exposure, where metabolism is saturated and the mixture components competitively inhibit each other's metabolism, a WOE approach using the BINWOEs summarized in Table 26 could be implemented. These predicted interactions based on metabolic saturation, as summarized previously, are likely to only occur at very high (e.g., ≥ 100 -fold times the corresponding MRL or TTD values) exposure levels. The BINWOEs predict that for toxicities mediated through reactive metabolites (hepatic, renal, immunological, respiratory, developmental, and carcinogenic), the estimated hazard or risk is likely to be less than indicated by the endpoint-specific hazard index or the total cancer risk. For neurological effects (chloroform, trichloroethylene, and vinyl chloride), the estimated hazard is likely to be greater than indicated by the hazard index for that endpoint for mixtures where chloroform is a major component (due to the neurotoxicity of the parent compound), and indeterminate for mixtures where trichloroethylene is a major component (due to neurotoxicity of both parent compound and a metabolite) or where vinyl chloride is a major component (due to lack of mechanistic understanding). Due to the number of compounds metabolized via cytochrome P450 enzymes, there is also potential for other co-exposures to impact the toxicity of this mixture at high concentrations that saturate metabolism (e.g., other chlorinated hydrocarbons), inhibit microsomal mixed-function oxidases (MFOs) (e.g., carbamates), or induce microsomal MFOs (e.g., acetone) (ATSDR 2019, 2022c, 2024a, 2024b).

4. Conclusions

This interaction profile recommends the use of component-based approaches that assume additive joint toxic action in exposure-based assessments of possible noncancer or cancer health hazards from inhalation or oral exposure to mixtures of chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride resulting from water contamination near hazardous waste sites. This recommendation is based on the following factors: (1) there are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all four components; (2) PBPK/PD models have not yet been developed that would predict pertinent target doses of the components under scenarios involving exposure to mixtures of all four components; and (3) available information on toxic actions of the individual components indicates that joint actions of chloroform, 1,1-dichloroethene, trichloroethylene, and vinyl chloride on several toxicity targets are plausible, including hepatic, renal, immunological, respiratory, neurological, developmental effects, and cancer. With data on the individual components suggesting possible sites of joint toxic action, but no data available on the toxicity or behavior of the complete mixture, a default component-based approach is therefore recommended.

WOE analyses of available data on the joint toxic action of mixtures of these components indicate that the available scientific evidence suggests less-than-additive interactions among these components for most endpoints, but only at concentrations sufficiently high to saturate metabolism. For the neurological effects of chloroform, these same mechanisms of metabolic saturation would result in more available parent compound, and therefore an increased toxicity, and for the neurological effects of trichloroethylene and vinyl chloride, the impact of this mechanism is indeterminate. However, as these concentrations are unlikely to be achieved in exposures resulting from water near hazardous waste sites, it is recommended that additivity be generally assumed in exposure-based assessments of health hazards from exposure to mixtures of these components. The dose additivity approach to screening for potential noncancer health hazard involves the estimation of endpoint-specific hazard indexes using MRLs from the toxicological profiles and TTDs derived in this interaction profile. This approach is appropriate when the hazard quotients of at least two of the components are ≥ 0.1 (ATSDR 2018). The response additivity approach to cancer risk involves adding the chemical-specific risks for chloroform, trichloroethylene, and vinyl chloride.

Endpoint-specific hazard indexes (e.g., hazard indexes for hepatic effects) or cancer risks for the same duration (e.g., chronic) can be summed across routes to estimate the aggregate hazard or risk, if it is likely that the same individual or group of individuals would be exposed by both routes. If an endpoint-specific

hazard index is >1 , or the total cancer risk for these chemicals is $\geq 1 \times 10^{-4}$, then further evaluation is needed (ATSDR 2018), using biomedical judgment and community-specific health outcome data, and considering community health concerns (ATSDR 1992). For very high exposures, e.g., ≥ 100 -fold above the MRLs or TTDs, interactions may occur, and their impact can be determined using the WOE results, as summarized above.

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Appendix A. Background Information for Chloroform

This appendix was written based primarily on the *Toxicological Profile for Chloroform* (ATSDR 2024a). Primary references are cited for the reader's convenience in identifying pertinent studies. For additional information beyond what is presented here, the reader is referred to the toxicological profile.

A.1 Toxicokinetic

Following inhalation exposure, absorption of chloroform appears to be rapid and extensive. Numerous studies in humans have demonstrated that inhaled chloroform is absorbed into the blood and extra-respiratory tissues (Aggazzotti et al. 1993; Cammann and Hübner 1995; Lévesque et al. 1994; Nashelsky et al. 1995). Quantitative measurement of inhalation absorption fraction or rate is limited to one study. In six adult subjects inhaling chloroform present in shower water and protected from dermal exposure, Xu and Weisel (2005) estimated the percent chloroform absorbed as 71% (range of 40–80%) using a one-compartment model. Animal toxicity studies of inhaled chloroform have provided evidence for absorption, but quantitative estimates have not been reported (see ATSDR 2024a). A study of absorption of an oral dose of ¹³C-labeled chloroform (0.5 g in a gelatin capsule) in volunteers revealed that absorption was both rapid and complete, with nearly 100% of the dose absorbed and peak blood levels in 1 hour after exposure (Fry et al. 1972). Experiments in mice, rats, and monkeys indicate that oral doses (up to 60 mg/kg) of ¹⁴C-labeled chloroform in olive oil were almost completely absorbed, as indicated by an 80–96% recovery of radioactivity in expired air, urine, and carcass (Brown et al. 1974; Taylor et al. 1974). Absorption in mice and monkeys was rapid; the peak blood levels were reached 1 hour after oral administration of 60 mg/kg chloroform in olive oil. Oral absorption of chloroform from an aqueous vehicle has been shown to be more rapid than from an oil vehicle (Pereira 1994; Withey et al. 1983), although absorption is complete from both vehicles.

Due to its lipophilic character, chloroform accumulates to a greater extent in tissues of high lipid content. Following absorption, the relative concentrations of chloroform in various tissues generally decrease as follows: adipose tissue > brain > liver > kidney > blood. The chloroform levels in seven patients who died after excessive administration during chloroform anesthesia were: brain, 372–480 mg/kg; lungs, 355–485 mg/kg; and liver, 190–275 mg/kg tissue wet weight (Gettler and Blume 1931); chloroform levels in patients under anesthesia who died from other causes were: brain, 120–182 mg/kg; lungs, 92–145 mg/kg; and liver, 65–88 mg/kg tissue wet weight. After whole-body autoradiography to study the distribution of inhaled ¹⁴C-labeled chloroform in mice, most of the radioactivity was found in fat

immediately after exposure, while the concentration of radioactivity in the liver increased during the postanesthetic period, most likely due to covalent binding to lipid and protein in the liver (Cohen and Hood 1969). Radioactivity from ^{14}C -labeled chloroform was detected in the placenta and fetuses of mice shortly after inhalation exposure (Danielsson et al. 1986). Studies of distribution of chloroform in humans following oral exposure are not available. Following oral exposure in animal studies, distribution of chloroform appears to be similar to distribution following inhalation exposure, with the primary concentrations in lipophilic tissues (Brown et al. 1974; Pfaffenberger et al. 1980; Taylor et al. 1974; Take et al. 2010).

Metabolism of chloroform occurs primarily by cytochrome P450-dependent pathways, with CYP2E1 (ethanol-inducible) being the primary isozyme responsible (Wang et al. 1994). The initial reaction results in the formation of a reactive intermediate, which gives off hydrochloric acid to form phosgene, which is then free to react with cellular macromolecules (including GSH, proteins, and nucleic acids) or conjugate with water to form carbon dioxide and hydrochloric acid (Ade et al. 1994; Branchflower et al. 1984; Pohl et al. 1981; Smith et al. 1984; Stevens and Anders 1981). On the basis of pharmacokinetic results obtained in rats and mice exposed to chloroform by inhalation, and of enzymatic studies in human tissues *in vitro*, *in vivo* metabolic rate constants ($V_{\max}\text{C} = 15.7 \text{ mg/hour/kg}$, $K_m = 0.448 \text{ mg/L}$) were defined for humans (Corley et al. 1990). Interspecies differences in the rate of chloroform conversion were observed in mice, rats, and squirrel monkeys, with species differences in metabolism being highly dose-dependent. The conversion of chloroform to carbon dioxide was highest in mice (80%) and lowest in squirrel monkeys (18%) (Brown et al. 1974). Similarly, chloroform metabolism was calculated to be slower in humans than in rodents.

Regardless of the route of exposure, chloroform is excreted from the body primarily as expired carbon dioxide, although at higher concentrations, where metabolism is saturated, appreciable levels of parent compound may be exhaled as well (Brown et al. 1974; Corley et al. 1990; Taylor et al. 1974). Only small amounts of chloroform or metabolites are excreted in the urine (Brown et al. 1974; Mink et al. 1986). The calculated biological half-time for chloroform in humans following inhalation exposure is on the order of 8 hours (Gordon et al. 1988). Nearly all of a single inhaled dose of chloroform is eliminated within 48 hours in rats and mice (Corley et al. 1990). In humans given a single oral dose of chloroform, most of the dose was exhaled as parent compound and carbon dioxide (Fry et al. 1972). Very little was excreted in the urine. Results in mice and rats given single oral doses of chloroform (Brown et al. 1974; Mink et al. 1986; Taylor et al. 1974) were similar to those seen from single inhalation exposures.

Numerous PBPK models exist for chloroform in both humans and animals. While a detailed discussion of these models is beyond the scope of this document (a complete discussion of the models can be found in ATSDR 2024a), the models, in general, are structured as multicompartment models with up to eight compartments, not including arterial and venous blood, and inputs for inhalation, oral, and dermal exposure. Models have been developed in mice, rats, and humans (Chinery and Gleason 1993; Corley et al. 1990, 2000; Evans et al. 2020; Gearhart et al. 1993; Reitz et al. 1990) and have been used to predict blood and tissue concentrations for multiple routes of exposure.

A.2 Health Effects

Hepatic Effects. Chloroform inhalation has been demonstrated to induce hepatic effects in both humans and animals. Acute, high-dose inhalation exposure to chloroform, such as in chloroform anesthesia, has been shown to cause jaundice, necrosis, liver enlargement and tenderness, and increased sulfobromophthalein retention in humans (ATSDR 2024a). Workers exposed to 14–400 ppm chloroform for 1–6 months developed toxic hepatitis and other effects including jaundice, nausea, and vomiting, without fever (Phoon et al. 1983). Toxic hepatitis (with hepatomegaly, enhanced serum ALT, and serum AST activities, and hypergammaglobulinemia) was observed in workers exposed chronically to 2–205 ppm chloroform (Bomski et al. 1967). Exposure of swimmers to lower levels of chloroform (18–24 ppm) did not result in detectable hepatic changes (Aiking et al. 1994). Animal studies of inhaled chloroform have also identified hepatic effects as a sensitive target, including altered liver enzymes, fatty changes, centrilobular degranulation, and necrosis (ATSDR 2024a).

The liver is a primary target of oral chloroform toxicity in humans, with some evidence that suggests the damage may be reversible (Wallace 1950). Hepatic injury occurred in patients within 1–3 days following chloroform ingestion (Piersol et al. 1933; Schroeder 1965; Storms 1973), which included jaundice and liver enlargement and tenderness, as well as several altered blood biochemical parameters (increased ALT, AST, and lactate dehydrogenase (LDH) activities and increased bilirubin levels). At autopsy, fatty degeneration and extensive centrilobular necrosis were observed (Dettling et al. 2016; Piersol et al. 1933). Increased sulfobromophthalein retention indicated impaired liver function in an individual who ingested 21 mg/kg/day chloroform in a cough medicine for 10 years (Wallace 1950); the changes resolved after exposure was discontinued. Biochemical tests indicate that liver function in male and female humans was not affected by the use of mouthwash providing 0.96 mg/kg/day chloroform for ≤ 5 years (De Salva et al. 1975).

The liver is also a target organ for oral chloroform toxicity in animals. Following acute-duration oral doses of ≥ 34 mg/kg, hepatic effects included increased liver weight, fatty changes, and necrosis (ATSDR 2024a). A no-observed-adverse-effect level (NOAEL) of 26 mg/kg/day (4 days) was identified in mice (Larson et al. 1994). Liver effects in animals have been reported in numerous oral studies of intermediate duration (Chu et al. 1982a; Eschenbrenner and Miller 1945; Larson et al. 1995a). Hepatic changes from intermediate-duration oral studies have included increased liver weight, increased levels of liver enzymes in serum, histological changes in hepatocytes, increased cell proliferation, and necrosis (ATSDR 2024a). The early effects of oral chloroform exposure appear to be reversible (EPA 1980). The lowest intermediate-duration exposure at which hepatic effects were seen was 30 mg/kg/day, with a NOAEL of 15 mg/kg/day, in dogs (Heywood et al. 1979). Results of chronic-duration oral studies have also identified hepatic effects as a sensitive effect of chloroform exposure, with effects including altered liver enzymes, hyperplasia, fatty liver, and fibrosis (Heywood et al. 1979; NCI 1976a; Tumasonis et al. 1985, 1987); the lowest level at which chronic effects were seen was 15 mg/kg/day, the lowest exposure tested, in dogs (Heywood et al. 1979). Hepatic effects served as the basis for the derivation of acute-, intermediate-, and chronic-duration oral MRLs for chloroform (see Section A.4).

Renal Effects. Studies of the effects of inhaled chloroform in humans have not clearly identified the kidney as a sensitive target of chloroform toxicity, although acute-duration, high-dose exposure has been shown to result in renal effects (Aiking et al. 1994; Li et al. 1993; Royston 1924). Acute- and intermediate-duration animal inhalation studies have suggested renal effects of chloroform, particularly tubular cell proliferation and necrosis (ATSDR 2024a). Acute, high-dose oral exposure to chloroform in humans results in albuminuria, urinary casts, epithelial swelling, and fatty degeneration of kidney tubules (Piersol et al. 1933; Schroeder 1965), while similar urinary symptoms were seen in one subject who ingested 21 mg/kg/day chloroform in cough medicine for 10 years (Wallace 1950). No indications of renal effects were observed in humans who ingested estimated doses of 0.34–0.96 mg/kg/day chloroform in mouthwash for 5 years (De Salva et al. 1975). Acute-duration, high-dose animal studies of oral chloroform exposure have also identified renal effects, including cytoplasmic vacuolization, swelling, and necrosis of proximal tubule cells (ATSDR 2024a). Intermediate-duration animal studies have also identified renal changes, including increased kidney weight, inflammation, renal cell proliferation, and proximal tubular necrosis (ATSDR 2024a). The lowest lowest-observed-adverse-effect level (LOAEL) reported by ATSDR (2024a) for renal effects of intermediate-duration oral exposure was 27 mg/kg/day for increased incidences of both hyperplasia and atypical tubules in male rats given chloroform in their drinking water for 10 months (Hooth et al. 2002; McDorman et al. 2003a, 2003b). In chronic-duration oral studies in rodents, the lowest LOAEL for renal effects was 45 mg/kg/day based on increased

incidences of both tubular lumen dilation and cytoplasmic basophilia in the proximal tubule identified in rats given chloroform in their drinking water for 104 weeks (Nagano et al. 2006). In dogs, fat deposition in renal glomeruli was observed at a dose of 30 mg/kg/day chloroform for 7.5 years, but not at 15 mg/kg/day (Heywood et al. 1979).

Respiratory Effects. As presented in case reports, humans exposed to high levels of chloroform via inhalation exhibited depressed respiratory rates and/or respiratory arrest (Jayaweera et al. 2017; Storms 1973; Whitaker and Jones 1965), effects that were likely secondary to depression of the central nervous system (CNS). Results of animal studies indicate that the nasal epithelium and the underlying nasal bones are targets of chloroform toxicity in rodents following acute-, intermediate-, and chronic-duration inhalation exposures (ATSDR 2024a). The acute-, intermediate-, and chronic-duration inhalation MRLs for chloroform were based on nasal lesions in rodents following exposures for 4 days (Larson et al. 1996; Templin et al. 1996a), 13 weeks (Templin et al. 1996b), and 104 weeks (Yamamoto et al. 2002), respectively. Similar effects were observed following acute- and intermediate-duration gavage dosing in rodents (Dorman et al. 1997; Larson et al. 1995a; Templin et al. 1996a, 1996b). Chloroform-induced damage to the lower respiratory tract was generally only detected in animals following exposure to lethal concentrations (Bowman et al. 1978; Kasai et al. 2002; NCI 1976a), although there is limited evidence in mice of lung inflammatory responses following inhalation exposure to low chloroform concentrations (de Oliveira et al. 2015).

Neurological Effects. The neurological effects of high-dose inhaled chloroform are well-documented; chloroform was once used as an anesthetic in humans. Levels of 3,000–30,000 ppm were used to induce anesthesia (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965), while concentrations of \approx 40,000 ppm, if continued for several minutes, could result in death (Featherstone 1947). Concentrations $<$ 1,500 ppm are insufficient to induce anesthesia, while concentrations of 1,500–2,000 ppm cause light anesthesia (Goodman and Gilman 1980). Following occupational exposures to chloroform at 223–1,163 ppm, workers with long-term (mean of 5.4 years) employment self-reported slowness, decreased concentration, irritability, and depression, which were not reported by workers with short-term (mean of 15 months) employment service (Challen et al. 1958). In formal neurological testing, workers exposed to chloroform at a mean concentration of 6.04 ppm for an average of 7.8 years exhibited significant deficits in visual retention, simple visual reaction time, symbol-digit substitution, digit span, and pursuit aiming relative to controls (Li et al. 1993). Workers exposed to chloroform at a mean concentration of 2.79 ppm exhibited deficits in pursuit aiming only. Case reports of neurological effects following oral exposure to chloroform identified abolished reflexes, unconsciousness, and coma (ATSDR 2024a). Following

regaining consciousness, most patients recovered fully. One patient experienced mild cerebellar damage characterized by intention tremor and gait instability but full recovery occurred within two weeks (Storms 1973). CNS depression has been observed in inhalation and oral studies in animals (ATSDR 2024a), with overt signs generally seen only at very high exposure levels. The NOAEL and LOAEL values for neurobehavioral effects were 31.1 mg/kg/day (up to 90 days) and 100 mg/kg/day (60 days), respectively, as determined by a battery of behavioral tests in mice administered the chemical by gavage in aqueous emulphor (Balster and Borzelleca 1982).

Developmental Effects. Data on the developmental effects of chloroform in humans following inhalation exposure are limited to a single case-control study (Swartz et al. 2015a, 2015b); exposure to chloroform via ambient outdoor air was not associated with incidences of spina bifida. Results of epidemiological studies evaluating relationships between developmental effects and ingestion of chloroform in chlorinated water are mixed; some studies reported associations between chloroform exposure and impaired *in utero* growth (Botton et al. 2015; Grazuleviciene et al. 2011; Summerhayes et al. 2012; Sun et al. 2020) but are confounded by co-exposure to numerous other substances, while other studies reported no associations between chloroform exposure and neurodevelopmental outcomes (Villanueva et al. 2018) or birth defects (Grazuleviciene et al. 2013; Kaufman et al. 2018, 2020; Zaganjor et al. 2020).

Animal studies of chloroform inhalation have consistently identified developmental effects, including growth retardation, decreased crown-rump length, altered ossification, cleft palate, and fetal resorption (ATSDR 2024a), generally beginning at 30 ppm chloroform or greater. Oral exposure of rats to ≥ 316 mg/kg/day on gestation days (GDs) 6–15 resulted in decreased pup body weight and increased resorptions, but not in increased frequency of malformations (Ruddick et al. 1983; Thompson et al. 1974). The NOAEL and LOAEL values for decreased fetal weight were 50 and 126 mg/kg/day, respectively, on GDs 6–15 in the rat (Thompson et al. 1974). Thompson et al. (1974) also identified an oral LOAEL for decreased fetal weight of 20 mg/kg/day in rabbits dosed on GDs 6–18. The potential developmental toxicity of intermediate-duration oral exposure to chloroform is even less well-characterized in animals. No neurobehavioral effects were reported in offspring of mice treated with 31.1 mg/kg/day for 6–10 weeks (Burkhalter and Balster 1979). In a continuous breeding study in mice, F1 males had increased epididymal weights and degeneration of the epididymal epithelium and F1 females had increased liver weight and hepatocellular degeneration at 41 mg/kg/day for 105 days (NTP 1988a).

Cancer. One occupational study of workers with “substantial” exposure to chloroform identified an increased risk of pancreatic cancer but no associations with other forms of cancer (Christensen et al.

2013). Epidemiology studies suggest an association between cancer in humans and the consumption of chlorinated drinking water (Bove et al. 2007; Font-Ribera et al. 2018; Gao et al. 2014; Jones et al. 2019), but the results are not conclusive at this time. Such an association implicates chloroform because chloroform is a known animal carcinogen and is the predominant trihalomethane in chlorinated drinking water; however, it is important to note that some of the many chemicals produced in the process of water chlorination are highly mutagenic and/or carcinogenic, and human data have not been able to adequately control for these co-exposures.

In animals, chronic-duration inhalation exposure to chloroform produced renal tumors in mice (Yamamoto et al. 2002). Evidence of chloroform carcinogenicity is mixed following intermediate-duration oral exposure in animals, with some studies suggesting no increase in tumor formation following exposures of <52 weeks to <250 mg/kg/day (Klaunig et al. 1986; Stoner et al. 1986), but one study reported that a 30-day exposure to 594 mg/kg/day in mice resulted in increased formation of hepatomas (Eschenbrenner and Miller 1945). Chloroform has been shown to be carcinogenic in numerous chronic-duration animal studies, resulting in tumors of the liver and kidney (ATSDR 2024a). In general, studies of exposure levels ≥ 60 mg/kg/day resulted in increased incidence of tumors, while carcinogenicity at lower exposure levels was less clear.

A.3 Mechanisms of Action

Chloroform is widely distributed to many tissues of the body in laboratory animals and, presumably, in humans; however, many studies have demonstrated that chloroform does not tend to accumulate in the body for extended periods. Chloroform may accumulate to some degree in the body fat stores; however, it quickly partitions out of the fat and is excreted by the normal routes and mechanisms. The liver, respiratory tract, and kidneys are considered to be the target organs for chloroform toxicity in both humans and laboratory animals.

Chloroform is largely metabolized in many tissues (particularly the liver and kidney) to carbon dioxide in humans and animals (Brown et al. 1974; Corley et al. 1990; Fry et al. 1972; Liu et al. 2013). Chloroform metabolism is catalyzed by cytochrome P450, isozyme CYP2E1 in particular, initiating an oxidative cleavage of the C-H bond producing trichloromethanol. Trichloromethanol is unstable and is rapidly transformed to phosgene (COCl_2). Phosgene may react with water to form CO_2 , which can be exhaled by the lung or excreted in the urine as carbonate or bicarbonate, and hydrochloric acid. Phosgene can also react with other molecules such as cysteine, deplete hepatic GSH (Docks and Krishna 1976; Pohl et al.

1981), and form adducts with microsomal proteins (Corley et al. 1990). Experiments performed with a CYP2E1 inhibitor or CYP2E1 knockout mice have demonstrated that chloroform's toxic effects to nasal, hepatic, and renal tissues are mediated by metabolites (Constan et al. 1999).

Chloroform toxicity can be attributed to the presence of both the parent compound and the formation of phosgene in most instances of toxicosis. High doses of inhaled chloroform have been reported to cause death (due to respiratory depression), ataxia, narcosis, and CNS depression, and due to the direct effects of the parent compound. Lower doses of chloroform in the air, feed, or water, or administered by gavage, with variable exposure times, may induce toxicity due to the presence of the parent compound or to production of phosgene during metabolism. It appears that the metabolite is responsible for hepatocellular damage, resulting in the ultimate leakage of hepatic enzymes (AST, ALT, gamma-glutamyl transferase [GGT], etc.) into the serum and cellular damage/necrosis. The accumulation of chloroform in the renal cortex of mice with the subsequent metabolism to phosgene most likely contributes to the renal toxicity of chloroform seen in male mice. Tubular necrosis, calcification, nephritis, increased kidney weight, alterations in Na/K excretion, and other cellular anomalies were observed in response to one or both of these toxicants.

A.4 Health Guidelines

ATSDR (2024a) derived an acute-duration inhalation MRL of 0.001 ppm for chloroform, based on a NOAEL of 2 ppm (human equivalent concentration NOAEL [NOAEL_{HEC}] of 0.04 ppm) for respiratory effects (nasal lesions) in rats and mice exposed for 4 days (Larson et al. 1996; Templin et al. 1996a) and an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

ATSDR (2024a) derived an intermediate-duration inhalation MRL of 0.0008 ppm for chloroform, based on a LOAEL of 2 ppm for respiratory effects (nasal lesions) in rats exposed for 13 weeks (Templin et al. 1996b) and an uncertainty factor of 90 (3 for extrapolation from a LOAEL to a NOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability).

ATSDR (2024a) derived a chronic-duration inhalation MRL of 0.0004 ppm for chloroform, based on a LOAEL of 5 ppm (human equivalent concentration LOAEL [LOAEL_{HEC}] of 0.11 ppm) for respiratory effects (nasal lesions) in mice exposed for 104 weeks (Yamamoto et al. 2002) and an uncertainty factor of

300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability).

ATSDR (2024a) derived an acute-duration oral MRL of 0.3 mg/kg/day for chloroform, based on a NOAEL of 26 mg/kg/day in the drinking water for 4 days for hepatic effects (hepatic lesions) in mice (Larson et al. 1994) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2024a) derived an intermediate-duration oral MRL of 0.1 mg/kg/day for chloroform, based on a NOAEL of 15 mg/kg/day (adjusted NOAEL [NOAEL_{ADJ}] of 13 mg/kg/day) for hepatic effects (increased serum ALT) in dogs dosed with chloroform in a capsule 1 time/day, 6 days/week for 6 weeks (Heywood et al. 1979) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (2024a) derived a chronic-duration oral MRL of 0.02 mg/kg/day for chloroform, based on a 10% benchmark dose lower confidence limit (BMDL₁₀) of 2.15 mg/kg/day (adjusted benchmark lower confidence limit [BMDL_{ADJ}] of 1.84 mg/kg/day) for hepatic effects (fatty cysts) in dogs dosed with chloroform 6 days/week for 7.5 years (Heywood et al. 1979) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

EPA (2001) has not derived a reference concentration (RfC) for chloroform.

EPA (2001) derived a reference dose (RfD) of 0.01 mg/kg/day for chloroform based on a BMDL₁₀ of 1.2 mg/kg/day (BMDL_{ADJ} of 1.0 mg/kg/day) for hepatic effects in dogs dosed with chloroform 6 days/week for 7.5 years (Heywood et al. 1979) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

HHS's *Fifteenth Report on Carcinogens* (NTP 2021a) states that chloroform is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals.

Under EPA's *Proposed Guidelines for Carcinogen Risk Assessment* (EPA 1996), EPA (2001) determined that chloroform is *likely to be carcinogenic to humans* by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues.

IARC (1999) classifies chloroform as *possibly carcinogenic to humans* (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals.

A.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for chloroform in this mixture are hepatic, renal, respiratory, neurological, and developmental. For endpoints that are not the basis of the MRL, TTDS are derived below, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2024a) and, in particular, the Levels of Significant Exposure (LSE) tables.

Inhalation TTDS

The human equivalent concentration point of departure (POD_{HEC}) for an extra-respiratory effect is calculated by multiplying the duration-adjusted point of departure by the ratio of the blood:gas partition coefficients in animals and humans $[(Hb/g)_A / Hb/g)_H]$. Since the partition coefficient for chloroform in rodents is greater than in humans (see ATSDR 2024a), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation. Templin et al. (1998) identified a NOAEL of 5 ppm and a LOAEL of 17 ppm for increased relative liver weight and centrilobular swelling in mice exposed to chloroform for 6 hours/day, 5 days/week, for 7 weeks. Similar NOAEL and LOAEL values of 5 and 23–30 ppm, respectively, were identified based on hepatocytic swelling in mice exposed to chloroform for 6 hours/day, 5 days/week, for 13 weeks. The NOAEL of 5 ppm was duration-adjusted to 0.9 ppm for a continuous exposure scenario and converted to a $NOAEL_{HEC}$ of 0.9 ppm. Application of an uncertainty factor of 30 (3 for animal to human extrapolations with dosimetric adjustments and 10 for human variability) yields an intermediate-duration $TTD_{HEPATIC}$ of 0.03 ppm.

Renal Effects, Intermediate Inhalation. Larson et al. (1996) identified a LOAEL of 10 ppm for renal cell proliferation in the proximal convoluted tubules of male mice exposed to chloroform for 6 hours/day, 5 days/week, for 13 weeks; 10 ppm was the lowest concentration evaluated. Similarly, Templin et al. (1998) identified NOAEL and LOAEL values of 5 and 23 ppm, respectively, for cellular proliferation and regenerative lesions in the proximal convoluted tubule of mice exposed to chloroform for 6 hours/day, 5 days/week, for 13 weeks. Based on the similar study design, the NOAEL of 5 ppm was selected as the point of departure (POD) for the TTD. The NOAEL of 5 ppm was duration-adjusted to 0.9 ppm for a continuous exposure scenario and converted to a $NOAEL_{HEC}$ of 0.9 ppm. Application of an uncertainty

factor of 30 (3 for animal to human extrapolations for dosimetric adjustments and 10 for human variability) yields an intermediate-duration TTD_{RENAL} of 0.03 ppm.

Respiratory Effects, Intermediate Inhalation. The intermediate-duration inhalation MRL for chloroform is 0.0008 ppm based on respiratory effects.

Neurological Effects, Intermediate Inhalation. Adequate studies of the neurological effects of chloroform following intermediate-duration inhalation exposure are not available. Two studies (Larson et al. 1996; Templin et al. 1996b) evaluated chloroform's neurotoxicity in rodents following intermediate-duration exposures but failed to identify treatment-related effects. A chronic-duration TTD_{NEURO} of 0.006 ppm was derived based on impaired hand-eye coordination in workers exposed to 2.76 ppm for 1–15 years (Li et al. 1993). This TTD is higher than the intermediate-duration inhalation MRL and was adopted as the intermediate-duration TTD_{NEURO} .

Developmental Effects, Intermediate Inhalation. No intermediate-duration inhalation studies evaluated the developmental toxicity of chloroform. In an acute-duration study, Schwetz et al. (1974) reported a LOAEL of 30 ppm for delayed ossification and wavy ribs in the offspring of rats exposed for 7 hours/day on GDs 6–15. Since developmental toxicity is dependent on exposure during sensitive timepoints and not duration of exposure, results of the Schwetz et al. (1974) study were used to derive the intermediate-duration inhalation TDD for developmental effects. The LOAEL of 30 ppm was adjusted to 8.75 ppm for a continuous exposure scenario. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{DEVEL} of 0.03 ppm.

Hepatic Effects, Chronic Inhalation. Yamamoto et al. (2002) identified a NOAEL of 29.1 ppm and a less serious LOAEL of 85.8 ppm for fatty liver changes in mice exposed for 6 hours/day, 5 days/week, for 104 weeks. The NOAEL of 29.1 ppm was duration-adjusted to 5.2 ppm for a continuous exposure scenario and converted to a $NOAEL_{\text{HEC}}$ of 5.2 ppm. Application of an uncertainty factor of 30 (3 for animal to human extrapolations using a dosimetric adjustment and 10 for human variability) yields a chronic-duration TTD_{HEPATIC} of 0.2 ppm. As this is greater than the intermediate-duration TTD_{HEPATIC} of 0.03 ppm, the lower value of 0.03 ppm is adopted as the chronic-duration TTD_{HEPATIC} .

Renal Effects, Chronic Inhalation. Yamamoto et al. (2002) identified NOAEL and LOAEL values of 5 and 29.1 ppm, respectively, based on renal tubular lesions in male mice exposed for 6 hours/day,

5 days/week, for 104 weeks. The NOAEL of 5 ppm was duration-adjusted to 0.89 ppm for a continuous exposure scenario and converted to a NOAEL_{HEC} of 0.89 ppm. Application of an uncertainty factor of 30 (3 for animal to human extrapolations with dosimetric adjustment and 10 for human variability) yields a chronic-duration TTD_{RENAL} of 0.03 ppm.

Respiratory Effects, Chronic Inhalation. The chronic-duration inhalation MRL for chloroform is 0.0004 ppm based on respiratory effects.

Neurological Effects, Chronic Inhalation. Li et al. (1993) reported neurological effects, including impaired hand-eye coordination, in workers exposed to 2.79 ppm chloroform for 1–15 years. The LOAEL of 2.79 ppm was duration-adjusted for a continuous exposure scenario assuming that the workers were exposed 8 hours/day, 5 days/week, resulting in a LOAEL_{ADJ} of 0.66 ppm. An uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability) was applied to derive the TTD_{NEURO} of 0.006 ppm.

Developmental Effects, Chronic Inhalation. No chronic-duration inhalation studies evaluated the developmental toxicity of chloroform. Schwetz et al. (1974) reported a less serious developmental LOAEL of 30 ppm in rats exposed for 7 hours/day on GDs 6–15. Because developmental toxicity is dependent on exposure during sensitive timepoints and not duration of exposure, results of the Schwetz et al. (1974) study were used to derive the chronic-duration inhalation TDD for developmental effects. The LOAEL of 30 ppm was adjusted to 8.75 ppm for a continuous exposure scenario. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{DEVEL} of 0.03 ppm.

Oral TTDs

As discussed in ATSDR (2024a), rodents are more susceptible to chloroform's toxic effects via gavage or other bolus dosing than continual exposure via drinking water. This difference may be due to saturation of detoxification pathways following gavage dosing and/or adaptive changes following drinking water exposures at environmentally relevant concentrations. Therefore, results of drinking water studies are more relevant to human health effects resulting from environmental exposure levels and scenarios. For these reasons, the oral TTDs discussed below are derived solely from results of drinking water or capsule-based studies.

Hepatic Effects, Intermediate Oral. The intermediate-duration oral MRL for chloroform is 0.1 mg/kg/day, based on hepatic effects.

Renal Effects, Intermediate Oral. Only one intermediate-duration study identified renal effects of chloroform in animals exposed via drinking water: McDorman et al. (2003a) identified an increased incidence of atypical tubules and hyperplasia in the kidneys of Eker rats exposed to chloroform at 27 mg/kg/day for 10 months. Since the Eker rat is a model for hereditary renal cancer, the results of this study are not suitable to derive a TTD for renal effects. In additional intermediate-duration drinking water studies, no renal effects were identified in rats exposed to ≤ 106 mg/kg/day for 3 weeks (Larson et al. 1995b), ≤ 193 mg/kg/day for 28 days (Chu et al. 1982b), or ≤ 200 mg/kg/day for 90 days (Chu et al. 1982a; EPA 1980), or in mice exposed to ≤ 329 mg/kg/day for 3 weeks (Larson et al. 1994) or ≤ 435 mg/kg/day for 90 days (EPA 1980). Additionally, dogs dosed with ≤ 30 mg/kg/day via capsules for up to 42 weeks did not develop renal effects (Heywood et al. 1979). The highest dose tested for each of these studies is the NOAEL for renal effects. Since a free-standing NOAEL is not suitable for MRL or TTD development, the intermediate-duration oral MRL of 0.1 mg/kg/day is adopted as the TTD_{RENAL}.

Neurological Effects, Intermediate Oral. No intermediate-duration study identified neurological effects of chloroform in animals exposed via drinking water. No neurological effects were identified in rats exposed to ≤ 193 mg/kg/day for 28 days (Chu et al. 1982b) or ≤ 200 mg/kg/day for 90 days (Chu et al. 1982a). The highest dose tested for each of these studies is the NOAEL for neurological effects. Since a free-standing NOAEL is not suitable for MRL or TTD development, the intermediate-duration oral MRL of 0.1 mg/kg/day is adopted as the TTD_{NEURO}.

Developmental Effects, Intermediate Oral. No intermediate-duration oral studies evaluated the developmental toxicity of chloroform. The available acute-duration oral developmental toxicity studies administered chloroform via gavage. Therefore, the intermediate-duration oral MRL of 0.1 mg/kg/day is adopted as the TTD_{DEVEL}.

Hepatic Effects, Chronic Oral. The chronic-duration oral MRL for chloroform is 0.02 mg/kg/day, based on hepatic effects.

Renal Effects, Chronic Oral. Heywood et al. (1979) identified a NOAEL of 15 mg/kg/day and a LOAEL of 30 mg/kg/day for renal effects (fat deposition in the glomeruli) in dogs given chloroform in a capsule 6 days/week for 7.5 years. The NOAEL of 15 mg/kg/day was adjusted for intermittent exposure,

resulting in a $\text{NOAEL}_{\text{ADJ}}$ of 13 mg/kg/day. An uncertainty factor of 100 (10 for animal to human extrapolations and 10 for human variability) applied to the $\text{NOAEL}_{\text{ADJ}}$ results in a $\text{TTD}_{\text{RENAL}}$ of 0.1 mg/kg/day for chronic-duration oral exposure.

Neurological Effects, Chronic Oral. No chronic-duration oral study of sensitive endpoints for neurological effects was available for chloroform. Further, no chronic-duration oral studies evaluated neurological effects of chloroform in animals exposed via drinking water. No neurological effects were identified in dogs dosed with ≤ 30 mg/kg/day via capsule 6 days/week for 7.5 years (Heywood et al. 1979). Because the highest dose tested is the NOAEL for neurological effects and a free-standing NOAEL is not suitable for MRL or TTD development, the chronic-duration oral MRL of 0.02 mg/kg/day is adopted as the $\text{TTD}_{\text{NEURO}}$.

Developmental Effects, Chronic Oral. No chronic-duration oral studies evaluated the developmental toxicity of chloroform. The available acute-duration oral developmental toxicity studies administered chloroform via gavage. Therefore, the chronic-duration oral MRL of 0.02 mg/kg/day is adopted as the $\text{TTD}_{\text{DEVEL}}$.

Summary (TTD for Chloroform)

Intermediate-Duration Inhalation TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 0.03 \text{ ppm}$$

$$\text{TTD}_{\text{RENAL}} = 0.03 \text{ ppm}$$

$$\text{MRL}_{\text{RESP}} = 0.0008 \text{ ppm}$$

$$\text{TTD}_{\text{NEURO}} = 0.006 \text{ ppm}$$

$$\text{TTD}_{\text{DEVEL}} = 0.03 \text{ ppm}$$

Chronic-Duration Inhalation TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 0.03 \text{ ppm}$$

$$\text{TTD}_{\text{RENAL}} = 0.03 \text{ ppm}$$

$$\text{MRL}_{\text{RESP}} = 0.0004 \text{ ppm}$$

$$\text{TTD}_{\text{NEURO}} = 0.006 \text{ ppm}$$

$$\text{TTD}_{\text{DEVEL}} = 0.03 \text{ ppm}$$

Intermediate-Duration Oral TTDs:

MRL_{HEPATIC} = 0.1 mg/kg/day

TTD_{RENAL} = 0.1 mg/kg/day

TTD_{NEURO} = 0.1 mg/kg/day

TTD_{DEVEL} = 0.1 mg/kg/day

Chronic-Duration Oral TTDs:

MRL_{HEPATIC} = 0.02 mg/kg/day

TTD_{RENAL} = 0.1 mg/kg/day

TTD_{NEURO} = 0.02 mg/kg/day

TTD_{DEVEL} = 0.02 mg/kg/day

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Appendix B. Background Information for 1,1-Dichloroethene

This appendix was written based primarily on the *Toxicological Profile for 1,1-Dichloroethene* (ATSDR 2022c). Primary references are cited for the reader's convenience in identifying pertinent studies. For additional information beyond what is presented here, the reader is referred to the toxicological profile.

B.1 Toxicokinetics

No studies evaluating the absorption of 1,1-dichloroethene in humans following inhalation or oral exposure were located. Animal studies have demonstrated that 1,1-dichloroethene is rapidly absorbed following inhalation exposure (Dallas et al. 1983; McKenna et al. 1978b), being detectable in blood following as little as 2 minutes of exposure (Dallas et al. 1983), and is linear up to concentrations of 150 ppm (Dallas et al. 1983). Animal studies of oral exposure of 1,1-dichloroethene have similarly demonstrated rapid and near-complete absorption (Reichert et al. 1979). Doses of 1,1-dichloroethene ranging from 10 to 100 mg/kg were rapidly and almost completely absorbed from the gastrointestinal tract of rats and mice following oral administration in corn oil (Jones and Hathway 1978a; Putcha et al. 1986). Rapid absorption likewise occurred following oral administration of 200 mg/kg in an aqueous emulsion, as evidenced by the observation that the largest percentage of the dose was exhaled during the initial 15-minute period (Chieco et al. 1981). After oral administration to rats of 1,1-dichloroethene labeled with radioactive carbon (^{14}C), 81–99.8% of the administered radioactivity was recovered within 72 hours (Reichert et al. 1979), indicating very rapid and near-complete absorption.

No studies evaluating the distribution of 1,1-dichloroethene in humans following inhalation or oral exposure were located. Following inhalation exposure of rats to 10 or 200 ppm of ^{14}C -labeled 1,1-dichloroethene, the highest level of radioactivity was found in the liver and kidneys after 72 hours, with only very small amounts present in other tissues (McKenna et al. 1978b). Preferential accumulation of radioactivity was reported in the kidney and liver of rats exposed to 2,000 ppm radiolabeled 1,1-dichloroethene for 2 hours (Jaeger et al. 1977); fasted animals showed a higher accumulation of radiolabel than unfasted animals. 1,1-Dichloroethene was rapidly distributed to all tissues examined following a single oral dose of the ^{14}C -labeled compound to rats (Jones and Hathway 1978b). The highest amount of radioactivity was found in the liver and kidneys within 30 minutes of administration, although more general redistribution throughout the soft tissues of the body followed.

The metabolism of 1,1-dichloroethene following oral administration in rats has been extensively studied (Jones and Hathway 1978a, 1978b; McKenna et al. 1978a; Reichert et al. 1979). The primary biotransformation pathway is believed to involve the metabolism by CYP2E1 to a reactive epoxide, 1,1-dichloroethene oxide (Jones and Hathway 1978b; McKenna et al. 1977; Reichert et al. 1979). This metabolite may react with cellular molecules, may be conjugated to GSH, or may rearrange to chloroacetyl chloride and eventually to monochloroacetic acid. It is believed that metabolism of 1,1-dichloroethene is saturable based on studies demonstrating that at high exposure levels, a greater amount of unchanged compound is eliminated in the expired air (Dallas et al. 1983; Jones and Hathway 1978b; McKenna et al. 1978a, 1978b; Reichert et al. 1979).

Regardless of route of exposure, elimination of 1,1-dichloroethene is rapid and accomplished primarily in the form of metabolites in the urine, with elimination of the parent compound in the expired air becoming more prevalent as the exposure levels increase. At low doses (<150 ppm by inhalation or ≤ 1 mg/kg/day orally), very little (≤ 1) of the parent compound is eliminated in the expired air, while at higher concentrations, the percentage eliminated as the parent compound increases (Dallas et al. 1983; Jones and Hathway 1978b; McKenna et al. 1978a; Reichert et al. 1979).

D'Souza and Andersen (1988) reported a PBPK model for 1,1-dichloroethene in rats, based on the model for styrene developed by Ramsey and Andersen (1984). The model consists of four compartments (liver, slowly perfused, richly perfused, and fat) as well as blood, and contains inputs for both oral and inhalation exposure. Metabolism is assumed to occur in the liver compartment and consists of an initial oxidation followed by conjugation with GSH. Values for organ volume and blood flow were taken from previous modeling efforts (Gargas et al. 1986). The model simulations were optimized using data from McKenna et al. (1978b) and Jones and Hathway (1978a, 1978b). Models for species other than the rat are not available.

B.2 Health Effects

Hepatic Effects. Following both inhalation and oral exposure, the most sensitive effects of 1,1-dichloroethene appear to be on the liver. Numerous studies in animals have identified hepatic effects, including both biochemical changes (e.g., alterations in serum enzyme levels indicative of liver injury) and marked histological changes (e.g., midzonal and centrilobular swelling of liver, degeneration, and necrosis of hepatocytes). These effects have been reported at acute-duration exposure concentrations as low as 15 ppm for 23 hours/day for 5 days, or at higher concentrations for shorter durations (ATSDR

2022c). 1,1-Dichloroethene's hepatotoxic effects are greater in fasted animals compared to nonfasted animals administered the same dose for the same exposure time. The hepatotoxic effects of 1,1-dichloroethene following intermediate- or chronic-duration inhalation exposure in animals are similar to those described above for acute-duration exposure. Two chronic-duration inhalation studies of 1,1-dichloroethene in animals have reported similar hepatic changes (Lee et al. 1977; Quast et al. 1986), including fatty changes in the liver, but the studies provide only suggestive evidence because of the poor presentation of the data. Similar effects on the liver are seen when 1,1-dichloroethene is given orally, with acute-duration effects at doses of 25–100 mg/kg including changes in liver serum enzymes, bile canalicular injury, and histological changes in liver cells (ATSDR 2022c). Chronic-duration oral exposure studies in animals have identified minor hepatic effects at exposure levels between 9 and 20 mg/kg/day (Nitschke et al. 1983; Quast et al. 1983; Rampy et al. 1977); the chronic-duration oral MRL of 0.05 mg/kg/day for 1,1-dichloroethene is based on a BMDL₁₀ of 4.51 mg/kg/day for hepatocellular changes in rats exposed *in utero* and throughout adulthood (Humiston et al. 1978; Quast et al. 1983).

Renal Effects. Adverse effects have been observed in the kidneys of laboratory animals following acute-, intermediate-, and chronic-duration inhalation exposure to 1,1-dichloroethene. These effects are manifested as enzyme changes (decreases in kidney monooxygenase and epoxide hydrolase levels), tubular alterations (hemoglobinuria), gross changes (increase in organ weight), and histological changes (tubular swelling, degeneration, and necrosis) (ATSDR 2022c). Effects have been reported in animals exposed by inhalation acutely to 10–300 ppm or chronically to 25–75 ppm. Similar renal effects have been reported following acute-duration oral exposure to 200–400 mg/kg (Chieco et al. 1981; Jenkins and Andersen 1978), but no renal effects were noted in animals following intermediate-duration oral exposure to 25 mg/kg/day, an exposure level that did not produce any adverse effects (Quast et al. 1983) or chronic-duration oral exposure to 30 mg/kg/day, an exposure level that resulted in mild hepatic effects (Rampy et al. 1977).

Respiratory Effects. Results of high-quality inhalation toxicity studies in rodents indicate that the upper respiratory system may be a sensitive target for exposed humans. After repeated inhalation exposures to 1,1-dichloroethene at concentrations as low as 6.25–25 ppm, rats and mice exhibited increased lung weights; hyperostosis; chronic active inflammation; nasal turbinate atrophy; and/or olfactory epithelial mineralization, necrosis, atrophy, and/or metaplasia (NTP 2015). Rats appeared to be more sensitive than mice based on the results of intermediate-duration studies. The intermediate- and chronic-duration inhalation MRLs for 1,1-dichloroethene are based on necrosis of the rat olfactory epithelium.

Developmental Effects. Following inhalation exposure in mice, rats, and rabbits, 1,1-dichloroethene has been shown to produce effects on the developing organism, but generally only at exposure levels (15–160 ppm) that also produced maternal effects (EPA 1977; Murray et al. 1979); observed effects in the offspring included increased skeletal and soft tissue anomalies and fetal resorptions. One oral study of neural tube defects in human newborns after maternal exposure to 1,1-dichloroethene via contaminated water has been published (NJDH 1992a, 1992b), but it provided only suggestive evidence of an association of 1,1-dichloroethene with developmental effects. A single study reported no developmental effects from oral exposure of 40 mg/kg/day of 1,1-dichloroethene in rats, an exposure level that produced no effects (on body weight gain, liver weight, food or water consumption) in the dams (Murray et al. 1979). A 3-generation reproductive study in rats (Nitschke et al. 1983) reported no dose-related changes at up to 30 mg/kg/day in the limited pup indices that were investigated. Mild liver effects (hepatocellular fatty change) were reported in the adults at 9–30 mg/kg/day.

Neurological Effects. CNS depression has been observed in humans after acute exposure to high concentrations of 1,1-dichloroethene, typically $\geq 4,000$ ppm (ATSDR 2022c). Evidence of CNS toxicity has also been identified in experimental animals acutely exposed to 1,1-dichloroethene via inhalation at or above concentrations associated with lethality (ATSDR 2022c). Symptoms included lethargy, hunched posture, dyspnea, CNS depression, narcosis, and, eventually, death following exposure to $\geq 2,000$ ppm for 4 hours (Klimisch and Freisberg 1979; Zeller et al. 1979). Increased sympathetic activity leading to cardiac arrhythmia has been identified in rats exposed to 25,600 ppm for 10 minutes (Siletnik and Carlson 1974). No evidence of neurotoxicity was identified in experimental animals following oral exposure to 1,1-dichloroethene (ATSDR 2022c).

Cancer. Chronic-duration occupational exposure to 1,1-dichloroethene was not associated with the occurrence of angiosarcoma in rubber-plant workers (Waxweiler 1981). Similarly, no association was found between occupational exposure and cancer mortality in 1,1-dichloroethene production and polymerization plant workers (Ott et al. 1976). The carcinogenicity of 1,1-dichloroethene in laboratory animals following inhalation exposure has been evaluated in intermediate- and chronic-duration studies with rats, mice, and Chinese hamsters (ATSDR 2022c). Exposure concentrations of 1,1-dichloroethene in these studies ranged from 10 to 200 ppm. Of the long-term inhalation bioassays conducted in laboratory animals to date, Maltoni et al. (1985) identified increased incidences of renal, pulmonary, and mammary gland tumors in mice, and NTP (2015) reported increased incidences of nasal respiratory epithelium adenoma and mesothelioma in male rats and increased incidences of leukemia, mammary gland tumors, and thyroid (C-cell) tumors in female rats.

No studies were located regarding cancer in humans after oral exposure to 1,1-dichloroethene. A number of chronic-duration studies in rats and mice have evaluated the carcinogenicity of 1,1-dichloroethene by oral exposure at dose levels of 0.5–150 mg/kg/day (ATSDR 2022c); a trend toward increased incidence of malignant and nonmalignant tumors in 1,1-dichloroethene-treated animals has been reported (NTP 1982; Ponomarev and Tomatis 1980; Quast et al. 1983), but in the majority of cases, the increase in tumor frequencies has not been statistically significant. Reported tumor types have included meningiomas, mammary gland fibroadenomas and adenofibromas, and liver cell adenomas and carcinomas; tumor types have not been consistent across studies.

B.3 Mechanisms of Action

The toxicity of 1,1-dichloroethene is the result of biotransformation reactions and not to the parent compound (Andersen et al. 1978, 1980; Jaeger et al. 1977; Jones and Hathway 1978c).

1,1-Dichloroethene is initially oxidized by the hepatic cytochrome P450 system, primarily CYP2E1, resulting in the formation of reactive and electrophilic products such as epoxides, acyl chlorides, and halogenated aldehydes, which are responsible for the liver toxicity via alkylation of macromolecules (Forkert et al. 1986). These reactive intermediates form GSH S-conjugates by the action of GSTs located in the hepatic cytosol and microsomes. GSH S-conjugates that are primarily secreted from the hepatocytes into plasma and S-conjugates entering the circulation after reabsorption from the small intestine are ultimately delivered to the kidney where they undergo glomerular filtration (Dekant et al. 1989). In the kidney, GSH S-conjugates may be metabolized to the corresponding cysteine S-conjugate, which may be acetylated to form the corresponding mercapturic acid and excreted in the urine (Vamvakas and Anders 1990). However, cysteine S-conjugates may also be metabolized by β -lyase, an enzyme located in the renal proximal tubule cells; the resulting unstable thiols in turn yield electrophilic products whose interactions with macromolecules are associated with nephrotoxicity. In summary, GSH S-conjugate formation of nephrotoxic haloalkenes competes with hepatic cytochrome P450 for substrates. The relative extent of these reactions *in vivo* appears to be decisive for the initiation of adverse effects either in the liver (via oxidation products generated by P450 system) or in the kidney (via formation and renal processing of S-conjugates).

B.4 Health Guidelines

ATSDR (2022c) did not derive an acute-duration inhalation MRL for 1,1-dichloroethene.

ATSDR (2022c) derived an intermediate-duration inhalation MRL of 0.001 ppm for 1,1-dichloroethene based on a $BMCL_{10}$ of 1.59 ppm ($BMCL_{HEC}$ of 0.036 ppm) for nasal effects (necrosis of the olfactory epithelium) in rats exposed for 6 hours/day, 5 days/week for 14 weeks (NTP 2015). The $BMCL_{10}$ was adjusted to continuous duration exposure prior to conversion to a human equivalent concentration (HEC), and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans after dosimetric adjustment and 10 for human variability).

ATSDR (2022c) adopted the intermediate-duration inhalation MRL of 0.001 ppm as the chronic-duration inhalation for 1,1-dichloroethene. The intermediate-duration inhalation MRL was adopted for the chronic-duration MRL because derivation of an MRL using chronic data would have resulted in an MRL which was higher than the intermediate-duration value.

ATSDR (2022c) did not derive an acute-duration oral MRL for 1,1-dichloroethene due to the limited oral gavage database and lack of acute dietary and drinking water exposure studies.

ATSDR (2022c) did not derive an intermediate-duration oral MRL for 1,1-dichloroethene due to the limited oral gavage database and lack of intermediate dietary and drinking water exposure studies.

ATSDR (2022c) derived a chronic-duration oral MRL of 0.05 mg/kg/day for 1,1-dichloroethene based on a $BMDL_{10}$ of 4.51 mg/kg/day in rats for hepatocellular changes in a 2-year exposure study (Humiston et al. 1978; Quast et al. 1983) and using an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for human variability).

EPA (2002) derived a chronic RfC of 0.2 mg/m³ (0.05 ppm) for 1,1-dichloroethene based on benchmark concentration analysis of hepatic effects (fatty liver) in a chronic-duration study in rats exposed to 25 or 75 ppm for 6 hours/day, 5 days/week (Quast et al. 1986) and an uncertainty factor of 30 (3 for animal to human extrapolation using dosimetric adjustment and 10 for human variability).

EPA (2002) derived a chronic RfD of 0.05 mg/kg/day for 1,1-dichloroethene based on benchmark dose (BMD) analysis of hepatic effects (fatty liver) in a chronic-duration study in rats (Humiston et al. 1978; Quast et al. 1986) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

HHS's *Fifteenth Report on Carcinogens* (NTP 2021b) does not list 1,1-dichloroethene as a carcinogen.

EPA (2002) concluded that 1,1-dichloroethene exhibits *suggestive evidence* of carcinogenicity but not sufficient evidence to assess human carcinogenic potential following inhalation exposure in studies in rodents. Additionally, EPA (2002) noted “the data for 1,1-dichloroethene are *inadequate* for an assessment of human carcinogenic potential by the oral route.” EPA (2002) has not performed quantitative assessments of carcinogenic potential for 1,1-dichloroethene for either the oral or inhalation route.

IARC (2019) classifies 1,1-dichloroethene, as vinylidene chloride, as *possibly carcinogenic to humans* (Group 2B) based on sufficient evidence in experimental animals.

B.5 Derivation of Target-Organ Toxicity Dose(s)

The endpoints of concern for 1,1-dichloroethene in this mixture are hepatic, renal, respiratory, and developmental effects. For endpoints that are not the basis of the MRL, TTDS are derived below, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2022c) and, in particular, the LSE tables.

Inhalation TTDS

The POD_{HEC} for an extra-respiratory effect is calculated by multiplying the POD by the ratio of the blood:gas partition coefficients in animals and humans $[(Hb/g)_A / (Hb/g)_H]$. Since information on the partition coefficients for 1,1-dichloroethene in humans was not available (EPA 2002), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation. NTP (2015) identified NOAEL and LOAEL values of 6.25 and 12.5 ppm, respectively, based on centrilobular cytoplasmic alterations in the liver of male rats exposed to 1,1-dichloroethene for 6 hours/day, 5 days/week for 14 weeks. The NOAEL was duration-adjusted to 1.1 ppm for a continuous exposure scenario and converted to a $NOAEL_{HEC}$ of 1.1 ppm. Application of an uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for human variability) yields a $TTD_{HEPATIC}$ of 0.04 ppm.

Renal Effects, Intermediate Inhalation. NTP (2015) identified a LOAEL of 6.25 ppm based on increased relative kidney weights in female mice exposed to 1,1-dichloroethene for 6 hours/day, 5 days/week for 14 weeks. The LOAEL was duration-adjusted to 1.1 ppm for a continuous exposure scenario and converted to a LOAEL_{HEC} of 1.1 ppm. Application of an uncertainty factor of 90 (3 for extrapolation from a minimal LOAEL to a NOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{RENAL} of 0.01 ppm.

Respiratory Effects, Intermediate Inhalation. The intermediate-duration inhalation MRL for 1,1-dichloroethene is 0.001 ppm is based on respiratory effects.

Neurological Effects, Intermediate Inhalation. No intermediate-duration studies evaluated the neurological effects of 1,1-dichloroethene. Acute-duration inhalation studies are limited to studies in rats and hamsters reporting clinical signs of neurotoxicity at concentrations ($\geq 2,000$ ppm) associated with lethality (Klimisch and Freisberg 1979; Zeller et al. 1979) and a study in rats reporting increased sympathetic activity leading to cardiac arrhythmia following a 10-minute exposure to 25,600 ppm (Siletnik and Carlson 1974). Available data on neurological effects are not a suitable basis for TTD derivation because they occurred only at or above exposure levels associated with significant mortality.

Developmental Effects, Intermediate Inhalation. No intermediate-duration inhalation studies evaluated the developmental toxicity of 1,1-dichloroethene. EPA (1977) reported incomplete ossification in the offspring of mice exposed to 15 ppm of 1,1-dichloroethene for 23 hours/day on GDs 6–16. Since developmental toxicity is dependent on exposure during sensitive timepoints and not duration of exposure, results of the EPA (1977) study were used to derive the intermediate inhalation TDD for developmental effects. The LOAEL of 15 ppm was duration-adjusted to 14 ppm for a continuous exposure scenario and converted to a LOAEL_{HEC} of 14 ppm. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{DEVEL} of 0.05 ppm.

Hepatic Effects, Chronic Inhalation. NTP (2015) identified a LOAEL of 25 ppm for chronic inflammation and diffuse fatty changes in the livers of rats exposed to 1,1-dichloroethene for 6 hours/day, 5 days/week, for 105 weeks. The LOAEL was duration-adjusted to 4.5 ppm for a continuous exposure scenario and converted to a LOAEL_{HEC} of 4.5 ppm. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{HEPATIC} of 0.02 ppm.

Renal Effects, Chronic Inhalation. NTP (2015) identified a LOAEL of 6.25 ppm based on an increased incidence of renal tubule hyperplasia in male mice exposed for 6 hours/day, 5 days/week for 105 weeks. The LOAEL was duration-adjusted to 1.1 ppm for a continuous exposure scenario and converted to a LOAEL_{HEC} of 1.1 ppm. Application of an uncertainty factor of 300 (10 for extrapolation from a LOAEL to a NOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) yields a TTD_{RENAL} of 0.004 ppm.

Respiratory Effects, Chronic Inhalation. The chronic-duration inhalation MRL for 1,1-dichloroethene is 0.001 ppm is based on respiratory effects.

Neurological Effects, Chronic Inhalation. No chronic-duration studies evaluated the neurological effects of 1,1-dichloroethene. Available acute-duration data on neurological effects (described above) are not a suitable basis for TTD derivation because they occurred only at or above exposure levels associated with significant mortality.

Developmental Effects, Chronic Inhalation. A TTD_{DEVEL} of 0.02 ppm is derived from the corresponding intermediate-duration value; see explanation in Chapter 3.

Oral TTDs

Hepatic Effects, Intermediate Oral. NTP (1982) identified a NOAEL for hepatic effects of 1,1-dichloroethene at 40 mg/kg/day, 5 days/week (adjusted to 29 mg/kg/day for continuous exposure) in 13-week studies in rats and mice. At the LOAELs of 100 mg/kg/day for both species, 1,1-dichloroethene produced hepatocytomegaly in male rats; fibrosis, bile duct hyperplasia, and pigmentation in female rats; and necrosis in male and female mice. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the NOAEL_{ADJ} yields a TTD_{HEPATIC} of 0.3 mg/kg/day.

Renal Effects, Intermediate Oral. 1,1-Dichloroethene has not been adequately tested for non-hepatic effects in intermediate-duration oral studies, but chronic-duration oral studies did not report renal effects at dose levels that caused mild hepatic effects. Thus, there are no dose-response data suitable for derivation of a TTD. The chemical, however, caused renal effects in animals in acute-duration oral studies and in acute- and intermediate-to-chronic-duration inhalation studies. Thus, the WOE for renal effects suggests that 1,1-dichloroethene would cause renal damage at higher doses than tested in

intermediate- and chronic-duration oral studies. The intermediate-duration oral $TTD_{HEPATIC}$ of 0.3 mg/kg/day can be adopted as the TTD_{RENAL} for intermediate-duration exposure.

Developmental Effects, Intermediate Oral. No developmental effects were reported in the only oral developmental toxicity study of 1,1-dichloroethene available, which tested a single exposure level, 40 mg/kg/day, in rats on GDs 6–15 (Murray et al. 1979). A 3-generation reproductive study in rats (Nitschke et al. 1983) reported no dose-related changes at up to 30 mg/kg/day in the limited pup indices (number of pups/litter, postnatal survival and body weight) that were investigated. Mild liver effects (hepatocellular fatty change) were reported in the adults at 9–30 mg/kg/day. Although there are no data to indicate that this endpoint is affected by the oral route, the available studies are not fully adequate and only one species, the rat, was tested. Data from the inhalation route indicate that 1,1-dichloroethene was developmentally toxic at exposures that also were maternally toxic and that the mouse was the most sensitive species. Given the possibility that 1,1-dichloroethene may also be developmentally toxic by the oral route, the intermediate-duration oral $TTD_{HEPATIC}$ of 0.3 mg/kg/day can be adopted as the TTD_{DEVEL} for chronic-duration exposure.

Hepatic Effects, Chronic Oral. The chronic-duration oral MRL of 0.05 mg/kg/day is based on hepatic effects.

Renal Effects, Chronic Oral. Chronic-duration oral studies in animals did not report renal effects at dose levels of 1,1-dichloroethene that caused mild hepatic effects, and this chemical has not been adequately tested for non-hepatic effects in intermediate-duration oral studies. Thus, there are no dose-response data suitable for derivation of a TTD. The chemical, however, caused renal effects in animals in acute-duration oral studies and in acute- and intermediate-to-chronic inhalation studies. Thus, the WOE suggests that 1,1-dichloroethene may cause renal damage at higher doses than tested in intermediate- and chronic-duration oral studies. The chronic-duration oral MRL of 0.05 mg/kg/day can be adopted as the TTD_{RENAL} for chronic-duration exposure.

Developmental Effects, Chronic Oral. No developmental effects were reported in the only oral developmental toxicity study of 1,1-dichloroethene available, which tested a single exposure level, 40 mg/kg/day, in rats on GDs 6–15 (Murray et al. 1979). A 3-generation reproductive study in rats (Nitschke et al. 1983) reported no dose-related changes at up to 30 mg/kg/day in the limited pup indices (number of pups/litter, postnatal survival, and body weight) that were investigated. Mild liver effects (hepatocellular fatty change) were reported in the adults at 9–30 mg/kg/day. Although there are no data

to indicate that this endpoint is affected by the oral route, the available studies are not fully adequate and only one species, the rat, was tested. Data from the inhalation route indicate that 1,1-dichloroethene was developmentally toxic at exposures that also were maternally toxic and that the mouse was the most sensitive species. Given the possibility that 1,1-dichloroethene may also be developmentally toxic by the oral route, the chronic-duration oral MRL of 0.05 mg/kg/day can be adopted as the TTD_{DEVEL} for chronic-duration exposure.

Summary (TTDs for 1,1-Dichloroethene)

Intermediate-Duration Inhalation TTDs:

$$TTD_{HEPATIC} = 0.04 \text{ ppm}$$

$$TTD_{RENAL} = 0.01 \text{ ppm}$$

$$MRL_{RESPIRATORY} = 0.001 \text{ ppm}$$

$$TTD_{DEVEL} = 0.05 \text{ ppm}$$

Chronic-Duration Inhalation TTDs:

$$TTD_{HEPATIC} = 0.02 \text{ ppm}$$

$$TTD_{RENAL} = 0.004 \text{ ppm}$$

$$MRL_{RESPIRATORY} = 0.001 \text{ ppm}$$

$$TTD_{DEVEL} = 0.02 \text{ ppm}$$

Intermediate-Duration Oral TTDs:

$$TTD_{HEPATIC} = 0.3 \text{ mg/kg/day}$$

$$TTD_{RENAL} = 0.3 \text{ mg/kg/day}$$

$$TTD_{DEVEL} = 0.3 \text{ mg/kg/day}$$

Chronic-Duration Oral TTDs:

$$MRL_{HEPATIC} = 0.05 \text{ mg/kg/day}$$

$$TTD_{RENAL} = 0.05 \text{ mg/kg/day}$$

$$TTD_{DEVEL} = 0.05 \text{ mg/kg/day}$$

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Appendix C. Background Information for Trichloroethylene

This appendix was written based primarily on the *Toxicological Profile for Trichloroethylene* (ATSDR 2019). For additional information beyond what is presented here, the reader is referred to the toxicological profile. Regarding the weight of evidence for, and human relevance of, fetal cardiac malformations observed in rats, the reader is referred to the updated conclusions reported in the *Targeted Systemic Evidence Map (SEM) and Rapid Systematic Review for Trichloroethylene and Developmental Cardiotoxicity* (ATSDR 2025). Primary references from these ATSDR publications are cited for the reader's convenience in identifying pertinent studies.

C.1 Toxicokinetics

Studies of humans and rats indicate that inhaled trichloroethylene is rapidly and efficiently absorbed (ATSDR 2019). Initial rates of uptake are high, but decrease as steady-state conditions are approached. Studies in humans indicated that 37–64% of inhaled trichloroethylene was absorbed. Ingested trichloroethylene is rapidly and completely absorbed by the gastrointestinal tract (ATSDR 2019). Fasted rats given gavage doses of 5–25 mg/kg trichloroethylene displayed peak blood concentrations within 6–10 minutes and absorbed >90% of the dose within 9 hours (D'Souza et al. 1985). Dermal absorption is rapid, as indicated by observations of peak blood and exhaled air concentrations occurring within 5 minutes after a human subject immersed one hand in a trichloroethylene solution (ATSDR 2019). Once absorbed, trichloroethylene is widely distributed to organs throughout the body (including the developing fetus) and, due to its lipophilic properties, can accumulate in fat to a limited degree (ATSDR 2019). For example, 17 hours after a 6-hour/day, 4-day exposure of rats to 200 ppm, trichloroethylene was detected in perirenal fat and blood, but was not detected in other tissues (Savolainen et al. 1977).

Studies of humans and rodents indicate that inhaled trichloroethylene is eliminated from the body predominantly in the urine as metabolites and, to a lesser degree, in exhaled breath as the parent chemical or other volatile metabolites such as trichloroethanol and carbon dioxide (ATSDR 2019). For example, following single or sequential daily exposures of human subjects to 50–380 ppm: 11 and 2% of the dose was eliminated as trichloroethylene and trichloroethanol, respectively, in exhaled breath; 58% was eliminated as urinary metabolites; and 30% was unaccounted for (Monster et al. 1976, 1979). Trichloroethylene was detected in exhaled breath of humans 18 hours after exposure ended due to the relatively long elimination half-life of trichloroethylene in fatty tissue (3.5–5 hours) compared with other tissues. Following inhalation exposure to radiolabeled trichloroethylene, mice excreted 75% of radioactivity in

the urine and 9% as carbon dioxide in exhaled breath (Stott et al. 1982). Similar patterns of elimination were observed in mice and rats following oral administration of trichloroethylene (Koizumi et al. 1986).

The principal urinary metabolites of trichloroethylene in humans and animals are trichloroethanol, trichloroethanol-glucuronide, and trichloroacetic acid (ATSDR 2019; Lash 2025; Lash et al. 2000). Trichloroethylene is principally metabolized in the liver, but metabolism can also occur in Clara cells of the lungs and in the kidney. The principal pathway in humans and animals involves initial oxidation catalyzed by cytochrome P450 isozymes (CYP2E1 and 2B1/2). It has been proposed that this reaction forms an epoxide intermediate (trichloroethylene oxide) that rapidly converts to chloral hydrate, but an alternative proposal indicates that chloral hydrate formation involves chlorine migration in an oxygenated trichloroethylene-CYP transition state (Lash et al. 2000). Chloral hydrate can be oxidized to trichloroacetic acid via chloral hydrate dehydrogenase or reduced to trichloroethanol via alcohol dehydrogenase. Trichloroacetic acid is a strong inducer of peroxisome proliferation and has been associated with hepatocarcinogenicity in rodents. Trichloroethanol can be conjugated with glucuronic acid via glucuronyl transferase to form trichloroethanol-glucuronide. Studies with human hepatic microsomes indicate that CYP2E1 is the predominant isozyme responsible for the initial steps in trichloroethylene metabolism, although there is evidence that cytochrome P450 isozymes induced by phenobarbital (i.e., CYP2B1/2) may also be involved. At high exposure levels, enzymes involved in the main oxidative metabolic pathway can be saturated, leading to conjugation with GSH to produce S-(1,2-dichlorovinyl) glutathione (DCVG). DCVG is acted on by GGT to remove glutamine, and the glycine is removed by the action of dipeptidases to yield S-(1,2-dichlorovinyl)-L-cysteine (DCVC). Cleavage of the cysteine by β -lyase in the kidney can lead to intermediates with reactive thiol groups that can react with cellular macromolecules leading to renal cytotoxicity and carcinogenicity. Sex-, strain-, and species-dependent differences in metabolism have been identified for trichloroethylene and need to be accounted for in the interpretation of animal data for human risk assessment (Lash 2025).

Minor metabolic pathways arising from the initial intermediate, trichloroethylene oxide, or the trichloroethylene-cytochrome P450 transition state include: (1) transformation to dichloroacetic acid through a dichloroacetyl chloride intermediate (this path appears to be more important in rodents than humans); (2) hydrolytic dechlorinations to form formic acid and carbon monoxide; and (3) hydrolytic dechlorinations to form carbon dioxide via, sequentially, glyoxylic acid chloride, glyoxylic acid, and oxalic acid (ATSDR 2019; Lash et al. 2000). Dichloroacetic acid can be conjugated with GSH followed by sequential removal of glutamine and glycine to form dichlorovinyl-cysteine. Dichlorovinyl-cysteine can be transported to the kidney, where cleavage by β -lyase produces an intermediate with a reactive thiol

group that can react with proteins and deoxyribonucleic acid (DNA), leading to kidney cytotoxicity and kidney tumor development. Another minor pathway that is possible via the cytochrome P450 isoenzyme metabolic path involves metabolism of trichloroethylene into trichloroacetaldehyde, which, instead of further oxidation to produce the choral hydrate, spontaneously reacts with the amino acid tryptamine to form trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) (Liu et al. 2025). While possible, there is limited evidence for formation of TaClo in humans following trichloroethylene exposure (Liu et al. 2025).

PBPK models have been developed for the disposition of trichloroethylene in mice, rats, and humans, including the prediction of target organ (e.g., liver, lung, brain, kidney) doses of biologically active metabolites (ATSDR 2019; Clewell et al. 2000; Fisher 2000; Lash 2025). The models are being used to aid assessment of noncancer and cancer human health risks based on rodent exposure-response data (Barton and Clewell 2000; Rhomberg 2000).

C.2 Health Effects

Hepatic Effects. Results of epidemiological studies indicate occupational populations exposed to trichloroethylene may develop hepatic effects including altered liver function indices and enlarged livers (ATSDR 2019); however, these findings are confounded by co-exposures to other chemicals, including solvents, and inadequate quantitative exposure assessments. Where quantitative exposure estimates were available, the trichloroethylene concentrations causing hepatotoxicity were generally greater than current-day occupational exposure limits. Evidence for trichloroethylene's hepatotoxicity in humans is stronger in case reports; patients exposed occupationally or non-occupationally presented with jaundice, hepatitis, hepatomegaly, hepatosplenomegaly, and/or liver failure (Ha et al. 2009; Huang et al. 2006; Jung et al. 2012; Kamijima et al. 2007; Xu et al. 2009).

In rodent studies, inhalation and oral trichloroethylene exposures produced dose-related increases in liver weight, increased plasma levels of liver-associated enzymes, inflammation and local immune response, and/or hepatocellular hypertrophy (ATSDR 2019; Lash 2025). A study with wild-type and CYP2E1 knockout mice indicated that CYP2E1-dependent metabolism of trichloroethylene was required to produce hepatotoxicity (Ramdhan et al. 2008). Relatively high exposure levels were necessary to induce hepatic effects in most animal studies.

Renal Effects. Limited epidemiological evidence points to the kidney as a target organ for trichloroethylene. Workers exposed to trichloroethylene and other chemicals in the workplace and renal

cancer patients with reported trichloroethylene exposures exhibited altered urinary protein levels (Bolt et al. 2004; Brüning et al. 1999; Carrieri et al. 2007; Selden et al. 1993). An increased risk for end-stage renal disease was identified for aircraft workers in a retrospective cohort study (Radican et al. 2006). No clear association between long-term exposure to trichloroethylene via drinking water and adverse renal effects has been reported (ATSDR 2019).

Results of acute inhalation toxicity studies in rats indicate trichloroethylene increases urinary proteins, glucosaminidase, γ -glutamyl transpeptidase, and glucose and serum urea nitrogen (ATSDR 2019). In longer-term inhalation studies, trichloroethylene increased kidney weights following intermediate- or chronic-duration exposures (Boverhof et al. 2013; Adams et al. 1951) and induced renal tubular megalonucleocytosis in male rats, but not female rats, following chronic-duration exposures (Maltoni et al. 1986, 1988). In intermediate-duration oral toxicity studies, trichloroethylene increased kidney weights, urinary protein and ketone levels, and incidences of renal tubular epithelial cell cytomegaly and karyomegaly (Berman et al. 1995; NTP 1990; Tucker et al. 1982). Oral dosing of rats and mice for chronic periods resulted in chronic nephropathy unlike that typically identified in aged animals (NCI 1976b; NTP 1988b, 1990).

Immunological Effects. Occupational exposure to trichloroethylene is associated with hypersensitivity-type reactions, scleroderma, decreased total lymphocyte counts and serum immunoglobulin G (IgG) and immunoglobulin M (IgM) levels, and modified serum cytokine levels (ATSDR 2019; Lash 2025). Inhalation exposures to trichloroethylene produced immunosuppression in animals including decreased splenic anti-sheep red blood cell (SRBC) IgM response and serum IgG levels in rats and mice, respectively (Boverhof et al. 2013; Kaneko et al. 2000). Results of oral studies in animals indicate that trichloroethylene enhances selected hypersensitivity or allergic reactions and may accelerate autoimmune responses (ATSDR 2019; Lash 2025). Keil et al. (2009) reported decreased thymus weights and increased numbers of activated T-cells in mice exposed via drinking water for 27–30 weeks.

Neurological Effects. As reviewed by ATSDR (2019), the nervous system has been identified as a toxicological target for trichloroethylene based on results of human and animal studies.

Trichloroethylene-induced neurological effects in humans include dizziness, headaches, sleepiness, nausea, and lethargy, and, at higher exposure levels, memory and reflex deficits, nerve damage, and unconsciousness. Experimental animal studies identified neurobehavioral effects following acute or repeated oral and inhalation exposures; these include impairment of visual, auditory, psychomotor, and cognitive function, neurochemical changes, neuropathy, and nerve cell morphological changes.

Several studies suggest that exposure to trichloroethylene is associated with Parkinson's disease in humans and the induction of Parkinson's disease-like symptoms in experimental animals, as reviewed by Lash (2025) and Liu et al. (2025). For example, inhalation exposures to trichloroethylene were shown to induce nigrostriatal dopaminergic neurodegeneration and motor and gait impairments, effects observed in Parkinson's disease patients, in rats exposed to 50 ppm for 8 weeks and mice exposed 100 ppm for 12 weeks (Adamson et al. 2023). Trichloroethylene-exposed rats are a proposed model of Parkinson's disease to test drug interventions using a battery of motor tests and biochemical assays to evaluate oxidative stress (Srivastava et al. 2024).

Developmental Effects. Epidemiological studies provide limited evidence for developmental effects, including spontaneous abortions, congenital heart disease, and low birth weight, following gestational trichloroethylene exposure; critical limitations of these studies include small sample sizes and/or co-exposures to other chemicals (ATSDR 2019). ATSDR (2025) concluded that there is inadequate evidence for developmental cardiotoxicity in humans following oral exposure and a low level of evidence following inhalation exposure in humans due to confounding via co-exposure to other chemicals, including other chlorinated solvents.

Trichloroethylene exposures to pregnant laboratory animals may induce adverse developmental effects, namely decreased fetal weights, perinatal survival, and litter size and incomplete skeletal ossification, these typically occur at maternally toxic exposure levels (ATSDR 2019). Developmental effects induced at non-maternally toxic doses across several studies include fetal heart malformations and alterations to the hippocampal brain region following dam exposures via drinking water. It should be noted that ATSDR (2025) concluded "developmental cardiotoxicity is not classifiable as a health effect in humans following inhalation or oral exposure to trichloroethylene." This conclusion is based on inadequate and low levels of evidence in animal studies following inhalation and oral exposure, respectively. Specifically, the oral exposure animal studies that reported development cardiotoxicity with trichloroethylene exposure (Dawson et al. 1993; Johnson et al. 2003) have critical design flaws, including the use of non-concurrent controls and exposure groups (Hardin et al. 2004; Johnson et al. 2004). Further, ATSDR (2025) reviewed evidence that mechanistic data do not provide sufficient support for an association between congenital heart defects and trichloroethylene exposure based on a systematic review conducted by Urban et al. (2020); ATSDR determined that mechanistic studies published since the systematic review were unlikely to alter its conclusions.

In a developmental immunotoxicity test, Peden-Adams et al. (2006) identified decreased plaque-forming cell (PFC) response to SRBCs in mouse pups born to dams exposed to trichloroethylene via drinking water during gestation and lactation. A review by Lash (2025) identified several studies that indicate that trichloroethylene elicits developmental immunotoxicity and that the placenta is a target organ.

Cancer. IARC (2014) concluded that there is sufficient evidence in humans and experimental animals for the carcinogenicity of trichloroethylene. Specifically, the weight of evidence is sufficient to conclude that trichloroethylene produces kidney tumors in humans; additionally, positive associations have been established between trichloroethylene exposure and increased incidences of liver cancer and non-Hodgkin lymphoma. Chronic-duration animal studies have shown that cancer can be caused by inhalation or oral exposure to trichloroethylene, but do not point to a single target organ for increased tumor incidence. Carcinogenic responses have been observed in the liver, kidney, testes, lymphatic system, and lung, but the observed responses are not consistent across studies of different species and strains of animals (ATSDR 2019).

In general, carcinogenic responses to trichloroethylene are thought to involve trichloroethylene metabolites (Bull 2000; Green 2000; Lash et al. 2000). This hypothesis is supported by observations that mice appear to be uniquely susceptible to trichloroethylene-induced liver and lung tumors and display higher rates of trichloroethylene metabolism than rats. In contrast, rats appear to be uniquely susceptible to trichloroethylene-induced kidney damage and tumors. Based on its review of available data, ATSDR (2019) concluded that there is adequate evidence to indicate that trichloroethylene is carcinogenic in mice, that the evidence for trichloroethylene carcinogenicity in rats is equivocal, and that further study is required to determine whether or not the processes that induce liver cancer in mice also operate in the human liver.

C.3 Mechanisms of Action

Like other solvents, nervous system effects from trichloroethylene are likely to involve disruption of neural membranes by the parent chemical, but trichloroethanol is also involved. In support of this hypothesis, Mikisková and Mikiska (1966) reported that i.p. administration of trichloroethanol was 5–6 times more potent than trichloroethylene in altering the electrical excitability of the motor cerebral cortex, threshold current intensity of electrical skin stimulation, and electroencephalogram and electrocardiograph results associated with CNS depression in guinea pigs. Blain et al. (1992) found that effects on visual evoked potentials in rabbits exposed to trichloroethylene by inhalation correlated better

with blood levels of trichloroethanol than trichloroethylene. A similar relationship was identified for electroretinographic measurements in rabbits exposed to trichloroethylene by inhalation (Blain et al. 1994).

Parkinson-like effects observed in animals exposed to trichloroethylene are attributed to degeneration of dopaminergic neurons in the substantia nigra (Lash 2025; Liu et al. 2025). Proposed underlying mechanisms include induction of leucine-rich repeat kinase 2 (LRRK2) activity, mitochondrial dysfunction, and oxidative stress (Lash 2025; Liu et al. 2025; Srivastava et al. 2024). Effects are likely due to a metabolite of trichloroethylene. One candidate is TaClo due to similarity in structure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound linked to Parkinson's disease (Liu et al. 2025). However, while animal studies have confirmed an association between TaClo exposure and development of Parkinson-like effects, direct evidence of TaClo formation in humans is limited (Liu et al. 2025).

As proposed for neurological effects, renal effects associated with trichloroethylene exposure have also been attributed to mitochondrial dysfunction and oxidative stress (Lash et al. 2025).

Metabolism of trichloroethylene is expected to produce cytotoxic and carcinogenic metabolites, including trichloroacetic acid, dichloroacetic acid, chloral hydrate, and 2-chloroacetaldehyde (ATSDR 2019).

Drinking water administration of trichloroacetic acid to rodents has produced carcinogenic changes in the liver that are associated with the proliferation of peroxisomes. Reactive oxygen species produced by peroxisomes are thought to be involved in a sequence of DNA damage, cytotoxicity, regenerative cell growth, and tumor development. Phenobarbital pretreatment and induction of hepatic cytochrome P450 isozymes appear to be associated with enhancement of acute trichloroethylene hepatotoxicity in rodents (Allemand et al. 1978; Moslen et al. 1977; Nakajima et al. 1990), providing further support for the idea that metabolites are responsible for the hepatotoxicity of trichloroethylene. Trichloroethylene-induced liver cancer and peroxisomal proliferation have been associated with rapid metabolism of trichloroethylene to trichloroacetic acid in mice, but this metabolic pathway appears to be limited in rats and humans. Dichloroacetic acid has also been shown to produce liver cancer in mice, but its hepatocarcinogenicity has been hypothesized to involve some other, as yet unspecified, mechanism of action. The mouse liver displays much higher rates of metabolism of trichloroethylene, and is more susceptible to the hepatotoxicity and hepatocarcinogenicity of trichloroethylene, than are the livers of rats and humans. With chronic-duration oral exposure to high doses of trichloroethylene by gavage, increased incidence of toxic nephrosis and renal tumors occurred in male rats, but in female rats, the nephrosis was not

accompanied by an increase in kidney tumors. Trichloroethylene-induced kidney damage has been proposed to involve conjugation products of trichloroethylene with GSH. The conjugated products (e.g., dichlorovinyl-cysteine) can be hydrolyzed by β -lyase in the kidney, forming a reactive thiol group that can react with cellular macromolecules and lead to cell damage. In support of this mechanistic hypothesis, chemical agents that inhibit β -lyase protected against dichlorovinyl-cysteine nephrotoxicity in rats (ATSDR 2019).

Trichloroethylene-induced cardiac arrhythmias are thought to involve parent-chemical sensitization of the heart to epinephrine-induced arrhythmias (ATSDR 2019). In animals, chemicals that inhibited the metabolism of trichloroethylene increased the potency of trichloroethylene to induce cardiac arrhythmias, whereas chemicals enhancing trichloroethylene metabolism decreased its potency.

C.4 Health Guidelines

ATSDR (2019) did not establish an acute-duration inhalation MRL for trichloroethylene.

ATSDR (2019) established intermediate- and chronic-duration inhalation MRLs of 0.0004 ppm for trichloroethylene based on EPA's inhalation RfC (see below for details) (EPA 2011).

ATSDR (2019) did not establish an acute-duration oral MRL for trichloroethylene.

ATSDR (2019) established intermediate- and chronic-duration oral MRLs of 0.0005 mg/kg/day for trichloroethylene based on EPA's oral RfD (see below for details) (EPA 2011).

EPA (2011) derived a chronic RfD of 0.0005 mg/kg/day for trichloroethylene based on the midpoint and rounding of three candidate critical study RfD values: increased incidence of fetal heart malformations in rats (Johnson et al. 2003), decreased thymus weights in female mice (Keil et al. 2009), and immunotoxicity (increased delayed-type hypersensitivity and decreased PFC response) in mice (Peden-Adams et al. 2006). EPA performed BMD modeling on the fetal heart malformation incidence in rats (Johnson et al. 2003) resulting in a BMDL₀₁ (1% extra risk) of 0.0207 mg/kg/day, which was used to calculate a PBPK model-based human equivalent dose (HED₉₉) of 0.0051 mg/kg/day. Dividing the HED₉₉ by a total uncertainty factor of 10 (accounting for extrapolation from animals to humans and human variability) resulted in a candidate RfD_{heart malformations} of 0.00051 mg/kg/day. For the thymus weight effects (Keil et al. 2009), EPA used the LOAEL of 0.35 mg/kg/day to derive a PBPK-model-based

HED₉₉ of 0.048 mg/kg/day. Dividing the HED₉₉ by a total uncertainty factor of 100 (accounting for extrapolation from a LOAEL to NOAEL and animals to humans and human variability) resulted in a candidate RfD_{thymus weight} of 0.00048 mg/kg/day. For immunotoxicity (Peden-Adams et al. 2006), EPA divided the LOAEL of 0.37 mg/kg/day by a total uncertainty factor of 1,000 (accounting for extrapolation from a LOAEL to a NOAEL and animals to humans, and human variability), producing a candidate chronic RfD_{immunotoxicity} of 0.00037 mg/kg/day. EPA selected 0.0005 mg/kg/day as the chronic RfD since it was the midpoint of the candidate RfDs following rounding to one significant figure. It should be noted that inclusion of developmental cardiotoxicity as a co-critical effect for the derivation of the RfD is considered controversial, and the quality of the Johnson et al. (2003) study has been challenged (ATSDR 2025). As discussed in ATSDR (2025), several evaluations determined that the database is insufficient to support an association between developmental cardiotoxicity and trichloroethylene exposure in humans (Bukowski 2014; EPA 2014; Wikoff et al. 2018), while others concluded that the weight of evidence indicates that trichloroethylene exposure may cause congenital heart defects (EPA 2020). Specifically, the rapid systematic review by ATSDR (2025) assigned the Johnson et al. (2003) study a low study confidence based on significant design flaws, including the lack of a concurrent control group.

EPA (2011) derived a chronic RfC of 0.0004 ppm for trichloroethylene based on the midpoint (rounded up) of candidate RfDs for two endpoints considered for the chronic RfD (see above): increased incidence of heart malformations in rats (Johnson et al. 2003) and decreased thymus weights in female mice (Keil et al. 2009). For heart malformations (Johnson et al. 2003), EPA performed BMD modeling and route-to-route extrapolation via a PBPK model to produce an HEC₉₉ of 0.0037 ppm. The HEC₉₉ was divided by a total uncertainty factor of 10 (accounting for extrapolation from animals to humans and human variability using a PBPK model), resulting in a candidate chronic RfC_{heart malformations} of 0.00037 ppm. For thymus weights (Keil et al. 2009), EPA performed route-to-route extrapolation via a PBPK model of the mouse LOAEL of 0.35 mg/kg/day to produce a HEC_{99LOAEL} of 0.033 ppm. Dividing the HEC_{99LOAEL} by a total uncertainty factor of 100 (accounting for extrapolation from a LOAEL to a NOAEL and animals to humans, and human variability using a PBPK model) produced a candidate RfC_{thymus weight} of 0.00033 ppm. EPA selected the midpoint of 0.0004 ppm (rounded up from 0.00035 ppm) as the chronic RfC for trichloroethylene. As noted above, both the critical study (Johnson et al. 2003) and the endpoint (developmental cardiotoxicity) have been heavily scrutinized for inclusion in the derivation of EPA RfDs.

NTP (2021c) listed trichloroethylene as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans. EPA (2011) concluded that trichloroethylene is *carcinogenic to humans* via a mutagenic mode of action according to the 2005 cancer guidelines (EPA 2005) based on

sufficient epidemiological evidence, and derived an oral slope factor of 4.6×10^{-2} per mg/kg-day and an inhalation unit risk of 4.1×10^{-6} per $\mu\text{g}/\text{m}^3$ for trichloroethylene. IARC (2014) assigned trichloroethylene to Cancer Group 1, *carcinogenic to humans*, based on sufficient evidence in humans and sufficient evidence in experimental animals.

C.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for trichloroethylene in this mixture are hepatic, renal, immunological, neurological, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2019) and, in particular, the LSE tables.

Inhalation TTDs

The POD_{HEC} for an extra-respiratory effect is calculated by multiplying the duration-adjusted animal POD by the ratio of the blood:gas partition coefficients in animals and humans $[(\text{Hb}/\text{g})\text{A} / (\text{Hb}/\text{g})\text{H}]$. Since the partition coefficients for trichloroethylene in rodents are greater than in humans, a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation. Kjellstrand et al. (1983) identified a NOAEL of 37 ppm and a LOAEL of 75 ppm for increased enzyme activity and liver weight in male mice exposed to trichloroethylene 24 hours/day for 30 days. The NOAEL was converted to a $\text{NOAEL}_{\text{HEC}}$ of 37 ppm as described previously under the heading, Inhalation TTDs. An uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for human variability) was applied to the $\text{NOAEL}_{\text{HEC}}$ to derive the $\text{TTD}_{\text{HEPATIC}}$ of 1 ppm.

Renal Effects, Intermediate Inhalation. Kjellstrand et al. (1983) reported NOAEL and LOAEL values of 37 and 75 ppm, respectively, for increased kidney weights in male mice exposed to trichloroethylene for 24 hours/day for 30 days. The NOAEL was converted to a $\text{NOAEL}_{\text{HEC}}$ of 37 ppm as described previously under the heading, Inhalation TTDs. An uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for human variability) was applied to the $\text{NOAEL}_{\text{HEC}}$ to derive the $\text{TTD}_{\text{RENAL}}$ of 1 ppm.

Immunological Effects, Intermediate Inhalation. EPA (2011) derived a chronic RfC for trichloroethylene based, in part, on decreased thymus weights in mice exposed to trichloroethylene in the drinking water at ≥ 1.4 ppm for 30 weeks (Keil et al. 2009) and route-to-route extrapolation. Since the intermediate-duration MRL for trichloroethylene is based on the EPA chronic RfC (ATSDR 2019), the intermediate-duration MRL of 0.0004 ppm can be adopted as the TTD_{IMMUNO} .

Neurological Effects, Intermediate Inhalation. Arito et al. (1994) reported a LOAEL of 50 ppm based on reduced wakefulness during exposure and postexposure sleeping heart rate in male mice exposed to trichloroethylene for 8 hours/day, 5 days/week for 6 weeks. The LOAEL was duration-adjusted to 11.9 ppm for a continuous exposure scenario and converted to a $LOAEL_{HEC}$ of 11.9 ppm as described previously under the heading, Inhalation TTDs. An uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) was applied to the $LOAEL_{HEC}$ to derive the TTD_{NEURO} of 0.04 ppm.

Developmental Effects, Intermediate Inhalation. While no single study has identified both a NOAEL and a LOAEL for developmental effects following inhalation of trichloroethylene, NIOSH (1980) and Hardin et al. (1981) identified a NOAEL of 500 ppm, while Dorfmueller et al. (1979) identified a LOAEL of 1,800 ppm for decreased fetal weight and incomplete skeletal ossification. ATSDR based its chronic-duration inhalation MRL on EPA's chronic RfC for trichloroethylene (EPA 2011). The chronic RfC is estimated as the midpoint (rounded up) of two candidate RfCs for different endpoints, one of which is developmental cardiotoxicity in rats following maternal exposure to trichloroethylene in drinking water (extrapolated to an inhaled concentration) on GDs 1–22 (Johnson et al. 2003). The chronic-duration inhalation MRL was adopted for an intermediate-duration inhalation MRL of 0.0004 ppm. Because the chronic-duration MRL is based on the EPA chronic RfC (ATSDR 2019), the chronic-duration MRL of 0.0004 ppm can be adopted as the TTD_{DEVEL} .

Hepatic Effects, Chronic Inhalation. A $TTD_{HEPATIC}$ of 0.3 ppm is derived from the intermediate-duration $TTD_{HEPATIC}$; see explanation in Chapter 3.

Renal Effects, Chronic Inhalation. Maltoni et al. (1988) reported a NOAEL of 100 ppm and a LOAEL of 300 ppm for renal tubule megalonucleocytosis in male rats exposed 7 hours/day, 5 days/week for 104 weeks. The NOAEL was duration-adjusted to 20.8 ppm for a continuous exposure scenario and converted to a $NOAEL_{HEC}$ of 20.8 ppm as described previously under the heading, Inhalation TTDs. An

uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment, 10 for human variability) was applied to the $\text{NOAEL}_{\text{HEC}}$ to derive the $\text{TTD}_{\text{RENAL}}$ of 0.7 ppm.

Immunological Effects, Chronic Inhalation. EPA (2011) derived a chronic RfC for trichloroethylene based in part on decreased thymus weights in rats exposed to trichloroethylene in the drinking water at ≥ 1.4 ppm for 30 weeks (Keil et al. 2009) and route-to-route extrapolation. Since the chronic-duration MRL for trichloroethylene is based on the EPA chronic RfC (ATSDR 2019), the chronic-duration MRL of 0.0004 ppm can be adopted as the $\text{TTD}_{\text{IMMUNO}}$.

Neurological, Chronic Inhalation. A $\text{TTD}_{\text{NEURO}}$ of 0.01 ppm is derived from the intermediate-duration $\text{TTD}_{\text{NEURO}}$; see explanation in Chapter 3.

Developmental Effects, Chronic Inhalation. EPA (2011) derived a chronic RfC for trichloroethylene based on the midpoint (rounded up) of candidate RfCs for two endpoints, including developmental cardiotoxicity in rats following maternal exposure to trichloroethylene via drinking water at ≥ 0.25 ppm on GDs 1–22 (Johnson et al. 2003) and route-to-route extrapolation. Because the chronic-duration inhalation MRL for trichloroethylene is based on the EPA chronic RfC (ATSDR 2019), the chronic-duration MRL of 0.0004 ppm can be adopted as the $\text{TTD}_{\text{DEVEL}}$.

Oral TTDs

Hepatic Effects, Intermediate Oral. The lowest intermediate-duration hepatic LOAEL of 400 mg/kg/day was reported by Buben and O’Flaherty (1985) based on enlarged hepatocytes in mice administered gavage doses of trichloroethylene 5 days/week for 6 weeks. The NOAEL for this study was 100 mg/kg/day; the duration-adjusted NOAEL is 71 mg/kg/day. Application of an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) applied to the $\text{NOAEL}_{\text{ADJ}}$ results in a TTD of 0.7 mg/kg/day for hepatic effects.

Renal Effects, Intermediate Oral. Tucker et al. (1982) reported a NOAEL of 217 mg/kg/day and a LOAEL of 393 mg/kg/day in male mice for elevated urinary protein and ketone levels from 6 months of exposure to trichloroethylene in the drinking water. Application of an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for human variability) results in a $\text{TTD}_{\text{RENAL}}$ of 2 mg/kg/day.

Immunological Effects, Intermediate Oral. EPA (2011) derived a chronic RfD for trichloroethylene based in part on decreased thymus weights in mice exposed to trichloroethylene via drinking water at ≥ 1.4 ppm for 30 weeks (Keil et al. 2009) and decreased PFC response and increased delayed-type sensitivity in pups exposed *in utero* via drinking water at ≥ 1.4 ppm (Peden-Adams et al. 2006). Since the intermediate-duration oral MRL for trichloroethylene is based on the EPA chronic RfD (ATSDR 2019), the intermediate-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{IMMUNO}.

Neurological Effects, Intermediate Oral. The lowest intermediate-duration neurological LOAEL of 1,000 mg/kg/day was identified by Gash et al. (2008) for decreased dopaminergic neurons in the substantia nigra brain region of male rats administered gavage doses of trichloroethylene 5 days/week for 6 weeks. A NOAEL was not identified for this study. Due to the nature of the critical effect, the dose of 1,000 mg/kg/day is designated as a serious LOAEL, which is not suitable for derivation of TTDs per ATSDR guidance. As the other neurological LOAELs identified for trichloroethylene are greater than the serious LOAEL of 1,000 mg/kg/day, they also are not suitable for TTD derivation. Since developmental toxicity and immunotoxicity, the effects for which the oral MRL is based, were detected at lower doses than those that cause neurotoxicity, the intermediate-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{NEURO}.

Developmental Effects, Intermediate Oral. EPA (2011) derived a chronic RfD for trichloroethylene based on the midpoint (rounded) of candidate RfDs for developmental cardiotoxicity in rats following maternal exposure to trichloroethylene at ≥ 0.25 ppm in drinking water on GDs 1–22 (Johnson et al. 2003) and decreased PFC response and increased delayed-type sensitivity in pups exposed *in utero* via drinking water at ≥ 1.4 ppm (Peden-Adams et al. 2006). Since the intermediate-duration oral MRL for trichloroethylene is based on the EPA chronic RfD (ATSDR 2019), the intermediate-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{DEVEL}.

Hepatic Effects, Chronic Oral. Chronic-duration studies of trichloroethylene toxicity have failed to report noncancer hepatic effects, even at doses as high as 1,000 mg/kg/day. The lowest intermediate-duration hepatic LOAEL of 400 mg/kg/day was reported by Buben and O’Flaherty (1985) based on enlarged hepatocytes in mice administered gavage doses of trichloroethylene 5 days/week for 6 weeks. The NOAEL value for this study was 100 mg/kg/day; the duration-adjusted NOAEL is 71 mg/kg/day. Application of an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) applied to the NOAEL_{ADJ} results in a TTD of 0.7 mg/kg/day for hepatic effects.

Renal Effects, Chronic Oral. Chronic-duration studies of trichloroethylene have reported kidney effects in rats and mice (Maltoni et al. 1986; NCI 1976b; NTP 1988b, 1990). The lowest LOAEL was 250 mg/kg/day based on an increased incidence of renal tubular nucleocytosis in male rats dosed 5 days/week for 52 weeks (Maltoni et al. 1986); the NOAEL was 50 mg/kg/day. Using the duration-adjusted NOAEL of 36 mg/kg/day and applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) results in a TTD_{RENAL} of 0.4 mg/kg/day.

Immunological Effects, Chronic Oral. EPA (2011) derived a chronic RfD for trichloroethylene based, in part, on decreased thymus weights in mice exposed to trichloroethylene via drinking water at ≥ 1.4 ppm for 30 weeks (Keil et al. 2009) and decreased PFC response and increased delayed-type sensitivity in pups exposed *in utero* via drinking water at ≥ 1.4 ppm (Peden-Adams et al. 2006). Since the chronic-duration MRL for trichloroethylene is based on the EPA chronic RfD (ATSDR 2019), the chronic-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{IMMUNO} .

Neurological, Chronic Oral. No chronic-duration oral studies evaluated neurological endpoints. The lowest intermediate-duration LOAEL of 1,000 mg/kg/day was identified by Gash et al. (2008) for decreased dopaminergic neurons in the substantia nigra brain region of male rats administered gavage doses of trichloroethylene 5 days/week for 6 weeks. A NOAEL was not identified for this study. Due to the nature of the critical effect, the dose of 1,000 mg/kg/day is designated as a serious LOAEL, which is not suitable for derivation of TTDs per ATSDR guidance. As the other intermediate-duration neurological LOAELs identified for trichloroethylene are greater than the serious LOAEL of 1,000 mg/kg/day, they also are not suitable for TTD derivation. Since developmental toxicity and immunotoxicity, the effects for which the chronic oral MRL is based, were detected at lower doses than those that cause neurotoxicity, the chronic-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{NEURO} .

Developmental Effects, Chronic Oral. EPA (2011) derived a chronic RfD for trichloroethylene based in part on increased incidence of fetal cardiac malformations in rats following maternal exposure to trichloroethylene at ≥ 0.25 ppm in drinking water on GDs 1–22 (Johnson et al. 2003) and decreased PFC response and increased delayed-type sensitivity in pups exposed *in utero* via drinking water at ≥ 1.4 ppm (Peden-Adams et al. 2006). Since the chronic-duration oral MRL for trichloroethylene is based on the EPA chronic RfD (ATSDR 2019), the chronic-duration MRL of 0.0005 mg/kg/day can be adopted as the TTD_{DEVEL} .

Summary (TTDs for Trichloroethylene)

Intermediate-Duration Inhalation TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 1 \text{ ppm}$$

$$\text{TTD}_{\text{RENAL}} = 1 \text{ ppm}$$

$$\text{MRL}_{\text{IMMUNO}} = 0.0004 \text{ ppm}$$

$$\text{TTD}_{\text{NEURO}} = 0.04 \text{ ppm}$$

$$\text{MRL}_{\text{DEVEL}} = 0.0004 \text{ ppm}$$

Chronic-Duration Inhalation TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 0.3 \text{ ppm}$$

$$\text{TTD}_{\text{RENAL}} = 0.7 \text{ ppm}$$

$$\text{MRL}_{\text{IMMUNO}} = 0.0004 \text{ ppm}$$

$$\text{TTD}_{\text{NEURO}} = 0.01 \text{ ppm}$$

$$\text{MRL}_{\text{DEVEL}} = 0.0004 \text{ ppm}$$

Intermediate-Duration Oral TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 0.7 \text{ mg/kg/day}$$

$$\text{TTD}_{\text{RENAL}} = 2 \text{ mg/kg/day}$$

$$\text{MRL}_{\text{IMMUNO}} = 0.0005 \text{ mg/kg/day}$$

$$\text{TTD}_{\text{NEURO}} = 0.0005 \text{ mg/kg/day}$$

$$\text{MRL}_{\text{DEVEL}} = 0.0005 \text{ mg/kg/day}$$

Chronic-Duration Oral TTDs:

$$\text{TTD}_{\text{HEPATIC}} = 0.7 \text{ mg/kg/day}$$

$$\text{TTD}_{\text{RENAL}} = 0.4 \text{ mg/kg/day}$$

$$\text{MRL}_{\text{IMMUNO}} = 0.0005 \text{ mg/kg/day}$$

$$\text{TTD}_{\text{NEURO}} = 0.0005 \text{ mg/kg/day}$$

$$\text{MRL}_{\text{DEVEL}} = 0.0005 \text{ mg/kg/day}$$

C.6 References

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Appendix D. Background Information for Vinyl Chloride

This appendix was written based primarily on the *Toxicological Profile for Vinyl Chloride* (ATSDR 2024b). Primary references are cited for the reader's convenience in identifying pertinent studies. For additional information beyond what is presented here, the reader is referred to the toxicological profile.

D.1 Toxicokinetics

Both human and animal studies have indicated a rapid absorption of vinyl chloride following inhalation exposure. For example, young adult male volunteers exposed to vinyl chloride concentrations of 2.9, 5.1, 11.7, or 23.5 ppm by gas mask for 6 hours (Krajewski et al. 1980) retained approximately 42% of the inhaled dose, regardless of concentration. Similar results have been reported in animal studies and have been incorporated into PBPK models for vinyl chloride (described below). While no studies of the absorption of vinyl chloride in humans are available, vinyl chloride is rapidly and completely absorbed following oral exposure in animals (Feron et al. 1981; Watanabe et al. 1976a; Withey 1976), with peak blood levels being reached 10–20 minutes after a single gavage dose (Withey 1976).

Studies of the disposition of vinyl chloride in humans are not available for any route of exposure. In animals, vinyl chloride is rapidly distributed following inhalation exposure, with highest levels in the kidney and brain (Bolt et al. 1976; Buchter et al. 1977). Unless metabolism is inhibited, vinyl chloride does not appear to deposit or accumulate for long periods within the body (Buchter et al. 1977). A similar pattern is seen following oral exposure (Watanabe et al. 1976a). Vinyl chloride can cross the placenta following absorption (Ungvary et al. 1978).

The major metabolic pathway of vinyl chloride involves oxidation by mixed-function oxidases, specifically CYP2E1, to form a highly reactive epoxide intermediate, 2-chloroethylene oxide, which spontaneously rearranges to form 2-chloroacetaldehyde (Guengerich et al. 1979, 1981; Gwinner et al. 1983; Laib 1982). These intermediates are detoxified mainly through conjugation with GSH catalyzed by GST enzymes. The conjugated products are excreted in urine as substituted cysteine derivatives and include thiodiglycolic acid, S-formyl-methylcysteine, and N-acetyl-S-(2-hydroxyethyl) cysteine (Bolt et al. 1980; Hefner et al. 1975). Metabolism is very rapid and is saturable (Bolt et al. 1977; Buchter et al. 1980; Filser and Bolt 1979; Watanabe et al. 1976a) at high exposure levels (~250 ppm by inhalation, and between 1 and 100 mg/kg by oral exposure).

Regardless of route of exposure, vinyl chloride is rapidly eliminated in the urine, primarily as metabolites. However, at very high concentrations when metabolism becomes saturated, elimination in the expired air may become a relevant pathway (Watanabe and Gehring 1976; Watanabe et al. 1976b).

Numerous PBPK models for vinyl chloride exposure have been published, for both inhalation and oral exposures; modeled species include rats, mice, hamsters, and humans. Several different modifications of these models have been used to estimate human cancer risk following vinyl chloride inhalation (Clewell et al. 1995, 2001; Reitz et al. 1996). The PBPK model described in Clewell et al. (2001) and in EPA (2000) was used to derive the chronic-duration oral MRL, based on exposures from the Til et al. (1983, 1991) dietary study. For additional details on PBPK models, see ATSDR (2024b).

D.2 Health Effects

Hepatic Effects. Following both inhalation and oral exposure, the most sensitive effects of vinyl chloride are on the liver. Numerous studies of workers exposed to atmospheres containing vinyl chloride have reported hepatic changes, including hepatic proliferation, hepatomegaly, fibrosis, and hepatocellular degeneration (ATSDR 2024b). While exposure characterization in these studies has been limited, effects have been reported at exposure levels ranging from 1 to 2,300 ppm (Ho et al. 1991; Suciú et al. 1975). The incidence and severity of the effects generally correlate well with the duration of exposure (Gedigke et al. 1975; Lilis et al. 1975; NIOSH 1977). Studies of humans following oral exposure to vinyl chloride are not available. Animal studies have identified noncancer hepatic effects beginning at inhaled concentrations of 10 ppm (Thornton et al. 2002) or oral doses of 1.7 mg/kg/day (Til et al. 1983, 1991). Chronic-duration exposure to vinyl chloride by inhalation has also been demonstrated to result in hepatic cancer, specifically angiosarcoma (ATSDR 2024b).

Immunological Effects. Workers exposed to vinyl chloride have shown a number of immunological effects, including “vinyl chloride disease” characterized by a syndrome consisting of Raynaud's phenomenon, acroosteolysis, joint and muscle pain, enhanced collagen deposition, stiffness of the hands, and scleroderma-like skin changes; these changes are thought to be immunologic in nature. Sera obtained from patients with varying degrees of severity of symptoms of vinyl chloride disease demonstrate a close correlation between the disease severity and the extent of the immunologic abnormality (Grainger et al. 1980; Langauer-Lewowicka et al. 1976; Ward 1976), although these symptoms have been reported without immunological findings (Black et al. 1986; Ostlere et al. 1992). In workers with severe clinical

signs, there have also been reports of an increased incidence of B-cell proliferation, hyperimmunoglobulinemia (Ward 1976), cryoglobulinemia (Grainger et al. 1980; Saad et al. 2017), and complement activation (Grainger et al. 1980; Saad et al. 2017; Ward 1976). Vinyl chloride workers who developed steatohepatitis (liver inflammation with fat accumulation) exhibited increased levels of proinflammatory cytokines (tumor necrosis factor- α , interleukins -1 β , -6, and -8) (Cave et al. 2010). Exposed workers were also found to have significantly increased percentages of lymphocytes compared to controls (Fučić et al. 1995, 1997). Evidence of a structurally altered IgG has been obtained, and it has been proposed that vinyl chloride or a metabolite binds to IgG (Grainger et al. 1980). Results of animal studies indicate that vinyl chloride inhalation exposure increases spleen weights (Bi et al. 1985; Sokal et al. 1980) and thymus weights (Sharma et al. 1980), induces spontaneous lymphocyte proliferation and, in mice, increases mitogen-stimulated responses (Sharma and Gehring 1979; Sharma et al. 1980) and increases the number of pulmonary interstitial macrophages (Zelko et al. 2022). No studies of immunological effects of oral exposure to vinyl chloride in humans or animals were located.

Neurological Effects. The most commonly reported CNS effects of vinyl chloride inhalation in humans are ataxia or dizziness, drowsiness or fatigue, loss of consciousness, and/or headache (ATSDR 2024b). Other CNS effects that have been reported by vinyl chloride workers include euphoria and irritability (Suciu et al. 1963, 1975), visual and/or hearing disturbances (Marsteller et al. 1975), nausea (Marsteller et al. 1975; Spirtas et al. 1975), memory loss (Langauer-Lewowicka et al. 1983; Suciu et al. 1963, 1975), and nervousness and sleep disturbances (Langauer-Lewowicka et al. 1983; Suciu et al. 1963). CNS tests revealed pyramidal signs and cerebellar disturbances in some exposed subjects (Langauer-Lewowicka et al. 1983). Reliable estimates of exposure levels producing these effects were not available, but they generally occur only at fairly high (>4,000 ppm) acute exposure levels (Lester et al. 1963; Patty et al. 1930). Chronic-duration inhalation exposure to lower levels of vinyl chloride may result in the development of a peripheral neuropathy characterized by tingling (paresthesia) in the extremities (Lilis et al. 1975; Sakabe 1975; Spirtas et al. 1975; Suciu et al. 1963, 1975; Veltman et al. 1975; Walker 1976), numbness in the fingers (Lilis et al. 1975; Sakabe 1975), weakness (Langauer-Lewowicka et al. 1983; Suciu et al. 1963, 1975), depressed reflexes (NIOSH 1977), warmth in the extremities (Suciu et al. 1963, 1975), and pain in the fingers (Sakabe 1975). However, it is unclear whether some of these symptoms are associated with tissue anoxia due to vascular insufficiency or whether they represent the direct toxic effects of vinyl chloride on peripheral nerves. Animal studies of inhaled vinyl chloride have also reported changes to nervous tissues, but generally only at very high (>5,000 ppm) exposure levels. No studies of neurological effects following oral exposure in humans or animals were located.

Developmental Effects. Although evidence has been presented indicating that members of communities with nearby vinyl chloride polymerization facilities have significantly greater incidences of some forms of developmental toxicity, these studies failed to demonstrate a statistically significant correlation between the developmental toxicity and either parental occupation or proximity to the facility (Edmonds et al. 1978; Infante et al. 1976; Rosenman et al. 1989; Theriault et al. 1983). Additionally, case-control studies have not identified associations between vinyl chloride exposure and developmental toxicity outcomes (Ruckart et al. 2013; Swartz et al. 2015a; Talbott et al. 2015). A number of inhalation studies have examined the effects of vinyl chloride exposure on pregnancy outcome in animals; results of these studies generally indicate that vinyl chloride produces adverse developmental effects (ATSDR 2024b). For example, John et al. (1977, 1981) reported a NOAEL of 50 ppm and a LOAEL of 500 ppm for maternal toxicity and delayed ossification in fetuses of mice and rabbits exposed during organogenesis, while Ungvary et al. (1978) reported that rats exposed to 1,500 ppm showed changes in maternal relative liver weights as well as increased litter resorption. Thornton et al. (2002) reported no adverse effects in rats following inhalation exposures to vinyl chloride at up to 1,100 ppm. No studies of developmental effects following oral exposure in humans or animals were located.

Cancer. The most compelling evidence for the carcinogenic potential of vinyl chloride in humans comes from the cluster of reports of greater than expected incidences of angiosarcoma of the liver in workers occupationally exposed to vinyl chloride (ATSDR 2024b). Angiosarcoma of the liver is considered to be a very rare type of cancer (25–30 cases/year in the United States) (Heath et al. 1975). However, approximately 30 years after the introduction of vinyl chloride for use in the industrial production of PVC, it became apparent that workers exposed to high levels of vinyl chloride had an unusually high incidence of this type of tumor. Other liver tumors, including hepatocellular carcinoma and cholangiocellular carcinoma, have also been associated with occupational exposure to vinyl chloride (ATSDR 2024b). Based on this information, vinyl chloride is considered to be a human carcinogen by IARC (2012), HHS (NTP 2021d), and EPA (EPA 2000). It has been suggested that inhalation exposure to vinyl chloride in humans may also result in increased incidences of cancers of the brain, CNS, respiratory tract, connective and other soft tissues, and lymphatic/hematopoietic systems (for additional details, see ATSDR 2024b); however, the evidence for these tumors is considerably less convincing than the evidence for hepatic tumors. No data on the carcinogenicity of vinyl chloride following oral exposure in humans were located. Studies in animals by both the inhalation and oral routes have confirmed the carcinogenic properties of vinyl chloride (ATSDR 2024b).

D.3 Mechanisms of Action

The majority of the proposed mechanisms of vinyl chloride toxicity involve the metabolism of the compound by CYP2E1 to a reactive intermediate, such as 2-chloroethylene oxide or 2-chloroacetaldehyde (Rusyn et al. 2021). The intermediary metabolites bind to macromolecules in the body. 2-Chloroethylene oxide is believed to bind primarily to DNA and ribonucleic acid (RNA), whereas 2-chloroacetaldehyde binds primarily to proteins (ATSDR 2024b). Modification of proteins may result in toxicity, as is believed to occur in vinyl chloride-induced liver lesions, or may alter their antigenicity, possibly resulting in the autoimmune responses associated with vinyl chloride exposure. The mechanisms resulting in the neurological effects of vinyl chloride are not well characterized.

Many studies have characterized the mutation profile associated with DNA adducts formed by the reactive metabolites of vinyl chloride (ATSDR 2024b). Four primary cyclic DNA etheno-adducts are formed by the reactive metabolites of vinyl chloride (1,N⁶-ethenoadenine, 3,N⁴-ethenocytosine, N^{2,3}-ethenoguanine, and 1,N²-ethenoguanine). These adducts can produce base-pair (i.e., purine-to-purine or pyrimidine-to-pyrimidine exchange) transitions during transcription (Cullinan et al. 1997; Pandya and Moriya 1996; Singer 1996; Singer et al. 1987). The role of etheno-adducts in the carcinogenesis of vinyl chloride has been recently reviewed (Guengerich and Ghodke 2021; Rioux and Delaney 2020). DNA crosslinks can also be formed because chloroacetaldehyde is bifunctional (Singer 1996). The mechanisms for clastogenic effects of vinyl chloride exposure were examined by Fučić et al. (1990); since chromatid and bichromatid breaks most frequently occurred in the terminal A, B, and C group chromosomes, these investigators suggested that vinyl chloride or its metabolites might interact with specific sites along the chromosome. Additionally, vinyl chloride induces cancer formation via epigenetic mechanisms including cell cycle disruption (Pan et al. 2021) and altering DNA methylation (Chappell et al. 2016).

D.4 Health Guidelines

ATSDR (2024b) derived an acute-duration inhalation MRL for vinyl chloride of 0.5 ppm based on a NOAEL of 50 ppm for developmental effects in mice exposed 7 hours/day on GDs 6–15 (John et al. 1977, 1981). The next higher exposure level, 500 ppm, produced mortality in the dams. The NOAEL of 50 ppm for intermittent exposure (7 hours/day) was converted to a continuous exposure (50 ppm x 7/24 = 15 ppm) and then converted to a HEC. Since the blood:gas partition coefficient for vinyl chloride in mice is greater than that in humans, a default value of 1 was used for the ratio resulting in a NOAEL_{HEC}

of 15 ppm. A total uncertainty factor of 30 (3 for extrapolation from mice to humans using a dosimetric adjustment and 10 for human variability) was applied to the $\text{NOAEL}_{\text{HEC}}$ to derive the MRL of 0.5 ppm.

ATSDR (2024b) derived an intermediate-duration inhalation MRL of 0.02 ppm for vinyl chloride based on a BMCL_{10} value of 2.05 ppm (BMCL_{HEC} of 0.5 ppm following adjustment to continuous duration and multiplication of the human to animal partition coefficient ratio default value of 1) for hepatic centrilobular hypertrophy in rats (Thornton et al. 2002) and a total uncertainty factor of 30 (3 for extrapolation from mice to humans using a dosimetric adjustment and 10 for human variability).

ATSDR (2024b) did not derive a chronic-duration inhalation MRL for vinyl chloride because of the absence of a suitable LOAEL or NOAEL for derivation.

ATSDR (2024b) did not derive acute- or intermediate-duration oral MRLs for vinyl chloride because of an absence of data on the effects of oral exposure to vinyl chloride for these duration categories.

ATSDR (2024b) derived a chronic-duration oral MRL of 0.003 mg/kg/day based on a NOAEL of 0.17 mg/kg/day for noncancerous liver effects (i.e., liver cell polymorphism) in rats (Til et al. 1983, 1991) and application of a PBPK model (Clewell et al. 2001; EPA 2000). The human model was run iteratively, until the model converged with the internal dose estimate for the rat (3.16 mg/L liver). The human dose was assumed to be uniformly distributed over a 24-hour period, with the resulting human equivalent dose (HED) of 0.09 mg/kg/day. Therefore, the $\text{NOAEL}_{\text{HED}}$ of 0.09 mg/kg/day, associated with the rat NOAEL of 0.17 mg/kg/day (Til et al. 1983, 1991), served as the basis for the chronic-duration oral MRL for vinyl chloride. A total uncertainty factor of 30 (3 for extrapolating from animals to humans using a dose metric conversion and 10 for human variability) was applied to the $\text{NOAEL}_{\text{HED}}$.

EPA (2000) derived a chronic RfD of 0.003 mg/kg/day for vinyl chloride using the same principal study, critical effect (hepatic changes), NOAEL, and PBPK model as described above for the chronic-duration oral MRL for vinyl chloride. A total uncertainty factor of 30 (3 for extrapolating from animals to humans using a dose metric conversion and 10 for human variability) was applied.

EPA (2000) derived a chronic RfC of 0.1 mg/m³ for vinyl chloride based on hepatic effects using a route-to-route extrapolation of the oral data from Til et al. (1983, 1991) using the Clewell et al. (2001) PBPK model. A total uncertainty factor of 30 (3 for extrapolating from animals to humans using a dose metric conversion and 10 for human variability) was applied.

HHS's *Fifteenth Report on Carcinogens* (NTP 2021d) reports that vinyl chloride is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans.

EPA has concluded that sufficient evidence of carcinogenicity exists in humans and animals and has classified vinyl chloride according to its 1986 classification scheme as a Group A or *known human carcinogen* (EPA 2000). EPA's current WOE characterization for vinyl chloride concludes that vinyl chloride is a *known human carcinogen* by the inhalation route of exposure, based on human epidemiological data. By analogy, vinyl chloride is considered a known human carcinogen by the oral route because of positive animal bioassay data as well as pharmacokinetic data allowing dose extrapolation across routes. By inference, vinyl chloride is also considered highly *likely to be carcinogenic* by the dermal route because it acts systemically (EPA 2000). Because the epidemiological evidence does not provide sufficient exposure and incidence data to quantify risk based solely on human data, EPA cancer potency factors for inhalation and oral exposure have been calculated based on animal studies. An inhalation unit risk of 8.8×10^{-6} per $\mu\text{g}/\text{m}^3$ for continuous lifetime exposure from birth was estimated by EPA (2000) based on the incidence of liver tumors observed in rats in the inhalation study by Maltoni et al. (1981). An inhalation unit risk of 4.4×10^{-6} per $\mu\text{g}/\text{m}^3$ for continuous lifetime exposure during adulthood was also estimated by EPA (2000). EPA (2000) derived the oral slope factor for continuous lifetime exposure as 1.5 per mg/kg/day based on the incidence of liver tumors in rats in the study by Feron et al. (1981). An oral slope factor of 7.5×10^{-1} per mg/kg/day for continuous lifetime exposure during adulthood was also estimated (EPA 2000).

IARC (2012) lists vinyl chloride in Group 1 (*carcinogenic to humans*) based on sufficient evidence of carcinogenicity in humans and animals.

D.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for vinyl chloride in this mixture are hepatic, immunological, neurological, and developmental. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2024b) and, in particular, the LSE tables.

Inhalation TTDs

The POD_{HEC} for an extra-respiratory effect is calculated by multiplying the duration-adjusted animal POD by the ratio of the blood:gas partition coefficients in animals and humans $[(Hb/g)_A / Hb(g)_H]$. Since the partition coefficients for vinyl chloride in rodents are greater than in humans (see ATSDR 2024b), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation. The intermediate-duration MRL for vinyl chloride is 0.02 ppm, based on hepatic effects.

Immunological Effects, Intermediate Inhalation. Zelko et al. (2022) reported a LOAEL of 0.8 ppm for increased pulmonary interstitial macrophages in mice exposed to vinyl chloride for 6 hours/day, 5 days/week for 12 weeks. The LOAEL was duration-adjusted to 0.14 ppm for a continuous exposure scenario and a $LOAEL_{HEC}$ of 0.14 ppm was calculated as described previously under the heading, Inhalation TTDs. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human extrapolation using a dosimetric adjustment, and 10 for human variability) would yield a TTD_{IMMUNO} of 0.0005 ppm. However, this would fall below the MRL; thus, the MRL of 0.02 ppm will be adopted as the TTD_{IMMUNO} for vinyl chloride.

Neurological Effects, Intermediate Inhalation. No appropriate data were available for intermediate-duration inhalation exposure. The intermediate-duration inhalation MRL of 0.02 ppm based on liver effects is adopted as the TTD_{NEURO} as a conservative value for intermediate-duration exposure.

Developmental Effects, Intermediate Inhalation. The acute-duration MRL of 0.5 ppm is based on developmental effects in mice exposed to 50 ppm of vinyl chloride for 7 hours/day (15 ppm $NOAEL_{HEC}$) on GDs 6–15, and is adopted as the TTD_{DEVEL} for intermediate-duration exposure.

Hepatic Effects, Chronic Inhalation. A $TTD_{HEPATIC}$ of 0.007 ppm was derived from the intermediate-duration MRL; see explanation in Chapter 3.

Immunological Effects, Chronic Inhalation. As for the intermediate-duration TTD_{IMMUNO} , the MRL of 0.02 ppm will be adopted as the chronic-duration TTD_{IMMUNO} for vinyl chloride.

Neurological Effects, Chronic Inhalation. No appropriate data were available for chronic-duration inhalation exposure. The intermediate-duration inhalation MRL of 0.02 ppm, based on liver effects, is adopted as the TTD_{NEURO} as a conservative value for chronic-duration exposure.

Developmental Effects, Chronic Inhalation. A TTD_{DEVEL} of 0.2 ppm was derived from the intermediate-duration value, using the approach explained in Chapter 3.

Oral TTDs

Hepatic Effects, Intermediate and Chronic Oral. No appropriate data were available for intermediate-duration oral exposure. The chronic-duration oral MRL of 0.003 mg/kg/day based on liver effects is adopted as a conservative value for intermediate-duration exposure.

Immunological Effects, Intermediate and Chronic Oral. No studies of immunological effects following oral exposure to vinyl chloride were located.

Neurological Effects, Intermediate and Chronic Oral. No appropriate data were available for intermediate-duration oral exposure. Feron et al. (1981) reported neurological NOAEL and LOAEL values of 5 and 14.1 mg/kg/day, respectively, based on lethargy, humpback position, and emaciation in rats administered gavage doses of vinyl chloride 5 days/week for 84 weeks to 2.7 years. The NOAEL was duration-adjusted to 3.6 mg/kg/day for continuous exposure. Application of an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for human variability) yields a TTD_{NEURO} of 0.04 mg/kg/day.

Developmental Effects, Intermediate and Chronic Oral. No studies of developmental effects following oral exposure to vinyl chloride were located.

Summary (TTDs for Vinyl Chloride)

Intermediate-Duration Inhalation TTDs:

$MRL_{HEPATIC} = 0.02$ ppm

$TTD_{IMMUNO} = 0.02$ ppm

$TTD_{NEURO} = 0.02$ ppm

$TTD_{DEVEL} = 0.5$ ppm

Chronic-Duration Inhalation TTDs:

TTD_{HEPATIC} = 0.007 ppm

TTD_{IMMUNO} = 0.02 ppm

TTD_{NEURO} = 0.02 ppm

TTD_{DEVEL} = 0.2 ppm

Intermediate- and Chronic-Duration Oral TTDs:

MRL_{HEPATIC} = 0.003 mg/kg/day (chronic), adopted as TTD_{HEPATIC} for intermediate

TTD_{IMMUNO} = not derived, no data

TTD_{NEURO} = 0.04 mg/kg/day

TTD_{DEVEL} = not derived, no data

D.6 References

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Appendix E. Database Query Strings for Combinations of Chloroform, 1,1-Dichloroethene, Trichloroethylene, and Vinyl Chloride

Information to prepare this profile was obtained via searches of the literature. The search objective was to identify noncancer and cancer toxicity, toxicokinetic, and interaction data from studies of humans and laboratory animals, as well as mechanistic studies using tissue, cell, or *in vitro* systems.

Initial searches of PubMed and Embase were conducted in September 2024 to identify references with records mentioning two or more of the four compounds of interest (chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride) using Chemical Abstracts Service Registry Numbers (CASRNs) and synonyms. Agency review documents were also collected for each of the compounds when available from IARC, EPA Integrated Risk Information System (IRIS) summaries and reviews, and National Toxicology Program (NTP). Table E-1 presents the CASRNs and names of the compounds, as well as synonyms used in the search. The ATSDR Toxicological Profiles for chloroform, 1,1-dichloroethylene, trichloroethylene, and vinyl chloride were consulted to identify CASRNs. Synonyms were generated by searching EPA's CompTox Chemicals Dashboard and Substance Registry Services and Chemical Abstracts Service (CAS) Common Chemistry database.

Table E-1. Substances Searched for Joint Toxic Action Studies in PubMed and Embase

Component	CAS Registry Number	Synonyms searched
Chloroform	67-66-3	1,1,1-trichloromethane; carbon trichloride; chloroform; chloroforme; chloroformium pro narcosi; cloroformio; cloroformo; formyl trichloride; freon 20; methane trichloride; methenyl chloride; methenyl trichloride; methyl trichloride; methylidyne trichloride; trichloormethaan; trichlormethan; trichloro-methane; trichloroform; trichloromethane; trichloromethyl radical; triclolorometano; (f 20 or f20) and freon; (r 20 or r20) and refrigerant
1,1-Dichloroethene	75-35-4	1,1-dce; 1,1-dichloraethen; 1,1-dichlorethylen; 1,1-dichlorethylene; 1,1-dichloroethene; 1,1-dichloroethylene; 1,1-dicloroetileno; as-dichloroethylene; asym-dichloroethylene; chlorure de vinylidene; diofan a 565s; f 1130a; hcc 1130a; hco 1130a; iso-dichloroethylene; r 1130a; vinylidene chloride; vinylidene dichloride; vinylidine chloride

Table E-1. Substances Searched for Joint Toxic Action Studies in PubMed and Embase

Component	CAS Registry Number	Synonyms searched
Trichloroethylene	79-01-6	1,1,2-trichloroethene; 1,1,2-trichloroethylene; 1,1-dichloro-2-chloroethylene; 1,2,2-trichloroethylene; 1-chloro-2,2-dichloroethylene; acetylene trichloride; algylen; anamenth; benzinol; blacosolv; blancosolv; cecolene; chlorilen; chlorylea; chlorylen; chorylen; circosolv; crawhaspol; densinfluat; dow-tri; dukeron; ethinyl trichloride; ethylene trichloride; f 1120; fleck-flip; flock flip; fluate; germalgene; hco 1120; lanadin; lethurin; lps hdx heavy duty degreaser; narcogen; narkosoid; per-a-clor; perm-a-chlor; petzinol; r 1120; tce (chlorohydrocarbon); threthylen; threthylene; trethylene; tri-clene; tri-plus; triasol; trichloorethen; trichloorethylene; trichloraethen; trichloraethylen; trichloraethylenum; trichloran; trichloren; trichlorethylen; trichlorethylene; trichlorethylenum; trichloroethene; trichloroethylene; trichloroethylenum; triciene; triclene; tricloretene; tricloroetilene; tricloroetileno; trielene; trielin; trielina; trieline; triklone n; trilen; trilene; trilene te-141; trimar; vestrol; vitran; westrosol
Vinyl chloride	75-01-4	1-chloroethene; 1-chloroethylene; chlorethene; chlorethylen; chlorethylene; chloroethene; chloroethylene; chlorure de vinyle; cloroetileno; cloruro de vinilo; cloruro di vinile; ethylene monochloride; f 1140; hco 1140; monochloroethene; monochloroethylene; monovinyl chloride; vinyl c monomer; vinyl chloride; vinylchloride

The query strings used for the literature searches are presented in Table E-2.

Table E-2. Database Query Strings

Database search date	Query string
PubMed 09/2024	((("Trichloroethylene"[mh] OR 79-01-6[rn] OR "1,1,2-Trichloroethene"[tw] OR "1,1,2-Trichloroethylene"[tw] OR "1,1-Dichloro-2-chloroethylene"[tw] OR "1,2,2-Trichloroethylene"[tw] OR "1-Chloro-2,2-dichloroethylene"[tw] OR "Acetylene trichloride"[tw] OR "Algylen"[tw] OR "Anamenth"[tw] OR "Benzinol"[tw] OR "Blacosolv"[tw] OR "Blancosolv"[tw] OR "Cecolene"[tw] OR "Chlorilen"[tw] OR "Chlorylea"[tw] OR "Chlorylen"[tw] OR "Chorylen"[tw] OR "CirCosolv"[tw] OR "Crawhaspol"[tw] OR "Densinfluat"[tw] OR "Dow-Tri"[tw] OR "Dukeron"[tw] OR "Ethinyl trichloride"[tw] OR "Ethylene trichloride"[tw] OR "F 1120"[tw] OR "Fleck-flip"[tw] OR "Flock FLIP"[tw] OR "Fluate"[tw] OR "Germalgene"[tw] OR "HCO 1120"[tw] OR "Lanadin"[tw] OR "Lethurin"[tw] OR "LPS HDX Heavy Duty Degreaser"[tw] OR "Narcogen"[tw] OR "Narkosoid"[tw] OR "Per-A-Clor"[tw] OR "Perm-A-chlor"[tw] OR

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	<p>"Petzino"[tw] OR "R 1120"[tw] OR "TCE (chlorohydrocarbon)"[tw] OR "Threthylene"[tw] OR "Threthylene"[tw] OR "Trethylene"[tw] OR "Tri-clene"[tw] OR "Tri-plus"[tw] OR "Triasol"[tw] OR "Trichlooretheen"[tw] OR "Trichloorethylene"[tw] OR "Trichloroethen"[tw] OR "TRICHLORAETHYLEN"[tw] OR "Trichloroethylenum"[tw] OR "Trichloran"[tw] OR "Trichloren"[tw] OR "Trichlorethylen"[tw] OR "Trichlorethylene"[tw] OR "Trichlorethyleneum"[tw] OR "TRICHLOROETHENE"[tw] OR "Trichloroethylene"[tw] OR "trichloroethylenum"[tw] OR "triciene"[tw] OR "Triclene"[tw] OR "Tricloretene"[tw] OR "Tricloroetilene"[tw] OR "tricloroetileno"[tw] OR "Trielene"[tw] OR "Trielin"[tw] OR "Trielina"[tw] OR "Trieline"[tw] OR "Triklone N"[tw] OR "Trilen"[tw] OR "Trilene"[tw] OR "Trilene TE-141"[tw] OR "Trimar"[tw] OR "Vestrol"[tw] OR "Vitran"[tw] OR "Westrosol"[tw]) AND ((75-35-4[rn] OR "vinylidene chloride"[nm] OR "1,1-DCE"[tw] OR "1,1-DICHLORAETHEN"[tw] OR "1,1-Dichlorethylen"[tw] OR "1,1-Dichlorethylene"[tw] OR "1,1-dichloroethene"[tw] OR "1,1-Dichloroethylene"[tw] OR "1,1-dicloroetileno"[tw] OR "as-Dichloroethylene"[tw] OR "asym-Dichloroethylene"[tw] OR "Chlorure de vinylidene"[tw] OR "Diofan A 565S"[tw] OR "F 1130a"[tw] OR "HCC 1130a"[tw] OR "HCO 1130a"[tw] OR "Iso-dichloroethylene"[tw] OR "R 1130a"[tw] OR "Vinylidene chloride"[tw] OR "Vinylidene dichloride"[tw] OR "Vinylidine chloride"[tw]) OR ("Vinyl Chloride"[mh] OR 75-01-4[rn] OR "1-Chloroethene"[tw] OR "1-Chloroethylene"[tw] OR "Chlorethene"[tw] OR "Chlorethylen"[tw] OR "Chlorethylene"[tw] OR "Chloroethene"[tw] OR "Chloroethylene"[tw] OR "chlorure de vinyle"[tw] OR "cloroetileno"[tw] OR "Cloruro de Vinilo"[tw] OR "Cloruro di vinile"[tw] OR "ethylene monochloride"[tw] OR "F 1140"[tw] OR "HCO 1140"[tw] OR "monochloroethene"[tw] OR "Monochloroethylene"[tw] OR "monovinyl chloride"[tw] OR "Vinyl c monomer"[tw] OR "Vinyl chloride"[tw] OR "Vinylchloride"[tw]) OR ("Chloroform"[mh] OR 67-66-3[rn] OR "1,1,1-Trichloromethane"[tw] OR "CARBON TRICHLORIDE"[tw] OR "Chloroform"[tw] OR "Chloroforme"[tw] OR "chloroformium pro narcosi"[tw] OR "Chloroformwith amylen"[tw] OR "Chloroformwith ethanol"[tw] OR "Cloroformio"[tw] OR "cloroformo"[tw] OR "Formyl trichloride"[tw] OR "Freon 20"[tw] OR "Methane trichloride"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloormethaan"[tw] OR "Trichlormethan"[tw] OR "Trichloro-Methane"[tw] OR "Trichloroform"[tw] OR "Trichloromethane"[tw] OR "Trichloromethyl radical"[tw] OR "Triclorometano"[tw] OR ((("F 20"[tw] OR "F20"[tw]) AND "freon**"[tw]) OR ((("R 20"[tw] OR "R20"[tw]) AND "refrigerant**"[tw]))) OR ((75-35-4[rn] OR "vinylidene chloride"[nm] OR "1,1-DCE"[tw] OR "1,1-DICHLORAETHEN"[tw] OR "1,1-Dichlorethylen"[tw] OR "1,1-Dichlorethylene"[tw] OR "1,1-dichloroethene"[tw] OR "1,1-Dichloroethylene"[tw] OR "1,1-dicloroetileno"[tw] OR "as-Dichloroethylene"[tw] OR "asym-Dichloroethylene"[tw] OR "Chlorure de vinylidene"[tw] OR "Diofan A 565S"[tw] OR "F 1130a"[tw] OR "HCC 1130a"[tw] OR "HCO 1130a"[tw] OR "Iso-dichloroethylene"[tw] OR "R 1130a"[tw] OR "Vinylidene chloride"[tw] OR "Vinylidene dichloride"[tw] OR "Vinylidine chloride"[tw]) AND ((("Vinyl Chloride"[mh] OR 75-01-4[rn] OR "1-Chloroethene"[tw] OR "1-Chloroethylene"[tw] OR "Chlorethene"[tw] OR "Chlorethylen"[tw] OR "Chlorethylene"[tw] OR "Chloroethene"[tw] OR "Chloroethylene"[tw] OR "chlorure de vinyle"[tw] OR "cloroetileno"[tw] OR "Cloruro de Vinilo"[tw] OR "Cloruro di vinile"[tw] OR "ethylene monochloride"[tw] OR "F 1140"[tw] OR "HCO 1140"[tw] OR "monochloroethene"[tw] OR "Monochloroethylene"[tw] OR "monovinyl chloride"[tw] OR "Vinyl c monomer"[tw] OR "Vinyl chloride"[tw] OR "Vinylchloride"[tw]) OR ("Chloroform"[mh] OR 67-66-3[rn] OR "1,1,1-Trichloromethane"[tw] OR "CARBON TRICHLORIDE"[tw] OR "Chloroform"[tw] OR "Chloroforme"[tw] OR "chloroformium pro narcosi"[tw] OR "Chloroformwith amylen"[tw] OR "Chloroformwith ethanol"[tw] OR "Cloroformio"[tw] OR "cloroformo"[tw])</p>

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Database search date	Query string
	<p>OR "Formyl trichloride"[tw] OR "Freon 20"[tw] OR "Methane trichloride"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloormethaan"[tw] OR "Trichlormethan"[tw] OR "Trichloro-Methane"[tw] OR "Trichloroform"[tw] OR "Trichloromethane"[tw] OR "Trichloromethyl radical"[tw] OR "Triclorometano"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND "freon**"[tw]) OR (("R 20"[tw] OR "R20"[tw]) AND "refrigerant**"[tw])) OR (("Vinyl Chloride"[mh] OR 75-01-4[rn] OR "1-Chloroethene"[tw] OR "1-Chloroethylene"[tw] OR "Chlorethene"[tw] OR "Chlorethylen"[tw] OR "Chlorethylene"[tw] OR "Chloroethene"[tw] OR "Chloroethylene"[tw] OR "chlorure de vinyle"[tw] OR "cloroetileno"[tw] OR "Cloruro de Vinilo"[tw] OR "Cloruro di vinile"[tw] OR "ethylene monochloride"[tw] OR "F 1140"[tw] OR "HCO 1140"[tw] OR "monochloroethene"[tw] OR "Monochloroethylene"[tw] OR "monovinyl chloride"[tw] OR "Vinyl c monomer"[tw] OR "Vinyl chloride"[tw] OR "Vinylchloride"[tw]) AND ("Chloroform"[mh] OR 67-66-3[rn] OR "1,1,1-Trichloromethane"[tw] OR "CARBON TRICHLORIDE"[tw] OR "Chloroform"[tw] OR "Chloroforme"[tw] OR "chloroformium pro narcosi"[tw] OR "Chloroformwith amylene"[tw] OR "Chloroformwith ethanol"[tw] OR "Cloroformio"[tw] OR "cloroformo"[tw] OR "Formyl trichloride"[tw] OR "Freon 20"[tw] OR "Methane trichloride"[tw] OR "Methenyl chloride"[tw] OR "Methenyl trichloride"[tw] OR "Methyl trichloride"[tw] OR "Methylidyne trichloride"[tw] OR "Trichloormethaan"[tw] OR "Trichlormethan"[tw] OR "Trichloro-Methane"[tw] OR "Trichloroform"[tw] OR "Trichloromethane"[tw] OR "Trichloromethyl radical"[tw] OR "Triclorometano"[tw] OR (("F 20"[tw] OR "F20"[tw]) AND "freon**"[tw]) OR (("R 20"[tw] OR "R20"[tw]) AND "refrigerant**"[tw])) AND (2004:3000[edat] OR 2004:3000[crdt] OR 2004:3000[mhda] OR 2004:3000[dp])</p>
Embase 09/2024	<p>Limit to (embase and yr="2004 -Current") ((Trichloroethylene/ or 79-01-6.rn. or (1,1,2-Trichloroethene or 1,1,2-Trichloroethylene or 1,1-Dichloro-2-chloroethylene or 1,2,2-Trichloroethylene or 1-Chloro-2,2-dichloroethylene or Acetylene trichloride or Algylen or Anamenth or Benzinol or Blacosolv or Blancosolv or Cecolene or Chlorilen or Chlorylea or Chlorylen or Chorylen or CirCosolv or Crawhaspol or Densinfluat or Dow-Tri or Dukeron or Ethinyl trichloride or Ethylene trichloride or F 1120 or Fleck-flip or Flock FLIP or Fluate or Germalgene or HCO 1120 or Lanadin or Lethurin or LPS HDX Heavy Duty Degreaser or Narcogen or Narkosoid or Per-A-Clor or Perm-A-chlor or Petzinol or R 1120 or TCE chlorohydrocarbon or Threthylen or Threthylene or Trethylene or Tri-clene or Tri-plus or Triazol or Trichlooretheen or Trichloorethylene or Trichloraethen or TRICHLORAETHYLEN or Trichloraethylenum or Trichloran or Trichloren or Trichlorethylen or Trichlorethylene or Trichlorethylenum or TRICHLOROETHENE or Trichloroethylene or trichloroethylenum or triciene or Triclene or Tricloretene or Trichloroetilene or trichloroetileno or Trielene or Trielin or Trielina or Trieline or Triklone N or Trilen or Trilene or Trilene TE-141 or Trimar or Vestrol or Vitran or Westrosol).ti,ab,kf.) and ((75-35-4.rn. or vinylidene chloride/ or (1,1-DCE or 1,1-DICHLORAETHEN or 1,1-Dichlorethylen or 1,1-Dichlorethylene or 1,1-dichloroethene or 1,1-Dichloroethylene or 1,1-dicloroetileno or asym-Dichloroethylene or Chlorure de vinylidene or "Diofan A 565S" or F 1130a or HCC 1130a or HCO 1130a or Iso-dichloroethylene or R 1130a or Vinylidene chloride or Vinylidene dichloride or Vinylidine chloride).ti,ab,kf.) or (Vinyl Chloride/ or 75-01-4.rn. or (1-Chloroethene or 1-Chloroethylene or Chlorethene or Chlorethylen or Chlorethylene or Chloroethene or Chloroethylene or chlorure de vinyle or cloroetileno or Cloruro de Vinilo or Cloruro di vinile or ethylene monochloride or HCO 1140 or monochloroethene or Monochloroethylene or monovinyl chloride or Vinyl c monomer or Vinyl chloride or</p>

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Database search date	Query string
	<p>Vinylchloride).ti,ab,kf.) or (Chloroform/ or 67-66-3.rn. or (1,1,1-Trichloromethane or CARBON TRICHLORIDE or Chloroform or Chloroforme or chloroformium pro narcosi or Chloroformwith amyleno or Chloroformwith ethanol or Cloroformio or cloroformo or Formyl trichloride or Freon 20 or Methane trichloride or Methenyl chloride or Methenyl trichloride or Methyl trichloride or Methylidyne trichloride or Trichloormethaan or Trichlormethan or Trichloro-Methane or Trichloroform or Trichloromethane or Trichloromethyl radical or Triclorometano or ((F 20 or F20) AND freon*) or ((R 20 or R20) AND refrigerant*).ti,ab,kf.)) or ((75-35-4.rn. or vinylidene chloride/ or (1,1-DCE or 1,1-DICHLORAETHEN or 1,1-Dichloroethylen or 1,1-Dichloroethylene or 1,1-dichloroethene or 1,1-Dichloroethylene or 1,1-dicloroetileno or asym-Dichloroethylene or Chlorure de vinylidene or "Diofan A 565S" or F 1130a or HCC 1130a or HCO 1130a or Iso-dichloroethylene or R 1130a or Vinylidene chloride or Vinylidene dichloride or Vinylidine chloride).ti,ab,kf.) and ((Vinyl Chloride/ or 75-01-4.rn. or (1-Chloroethene or 1-Chloroethylene or Chlorethene or Chlorethylen or Chlorethylene or Chloroethene or Chloroethylene or chlorure de vinyle or cloroetileno or Cloruro de Vinilo or Cloruro di vinile or ethylene monochloride or HCO 1140 or monochloroethene or Monochloroethylene or monovinyl chloride or Vinyl c monomer or Vinyl chloride or Vinylchloride).ti,ab,kf.) or (Chloroform/ or 67-66-3.rn. or (1,1,1-Trichloromethane or CARBON TRICHLORIDE or Chloroform or Chloroforme or chloroformium pro narcosi or Chloroformwith amyleno or Chloroformwith ethanol or Cloroformio or cloroformo or Formyl trichloride or Freon 20 or Methane trichloride or Methenyl chloride or Methenyl trichloride or Methyl trichloride or Methylidyne trichloride or Trichloormethaan or Trichlormethan or Trichloro-Methane or Trichloroform or Trichloromethane or Trichloromethyl radical or Triclorometano or ((F 20 or F20) AND freon*) or ((R 20 or R20) AND refrigerant*).ti,ab,kf.)) or ((Vinyl Chloride/ or 75-01-4.rn. or (1-Chloroethene or 1-Chloroethylene or Chlorethene or Chlorethylen or Chlorethylene or Chloroethene or Chloroethylene or chlorure de vinyle or cloroetileno or Cloruro de Vinilo or Cloruro di vinile or ethylene monochloride or HCO 1140 or monochloroethene or Monochloroethylene or monovinyl chloride or Vinyl c monomer or Vinyl chloride or Vinylchloride).ti,ab,kf.) and (Chloroform/ or 67-66-3.rn. or (1,1,1-Trichloromethane or CARBON TRICHLORIDE or Chloroform or Chloroforme or chloroformium pro narcosi or Chloroformwith amyleno or Chloroformwith ethanol or Cloroformio or cloroformo or Formyl trichloride or Freon 20 or Methane trichloride or Methenyl chloride or Methenyl trichloride or Methyl trichloride or Methylidyne trichloride or Trichloormethaan or Trichlormethan or Trichloro-Methane or Trichloroform or Trichloromethane or Trichloromethyl radical or Triclorometano or ((F 20 or F20) AND freon*) or ((R 20 or R20) AND refrigerant*).ti,ab,kf.))</p>