DRAFT

INTERACTION PROFILE FOR: CHLOROFORM, 1,1-DICHLOROETHYLENE, TRICHLOROETHYLENE, AND VINYL CHLORIDE

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

August 2007

PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandate 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 (NTP), 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 Division of Toxicology and Environmental Medicine (DTEM) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development 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 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.

CONTRIBUTORS

CHEMICAL MANAGER(S)/AUTHORS:

Hana Pohl, M.D., Ph.D.
ATSDR, Division of Toxicology and Environmental Medicine, Atlanta, GA

Mark Osier, Ph.D., D.A.B.T.
Syracuse Research Corporation, Syracuse, NY

Joan Colman, Ph.D.
Syracuse Research Corporation, Syracuse, NY

PEER REVIEW

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

1. Richard Hertzberg, Ph.D., Waste Management Division, U.S. Environmental Protection Agency, Atlanta, GA, USA /currently retired/
2. Kannan Krishnan, Ph.D., Human Toxicology Research Group, University of Montreal, Montreal, PQ, Canada
3. Scott Masten, Ph.D., Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

All reviewers were selected in conformity with the conditions for peer review specified in CERCLA Section 104(I)(13).

Scientists from ATSDR have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this mixture. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

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-dichloroethylene, 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 1994, 1997a, 1997b, 2004b); 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-dichloroethylene, 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, 2004a) 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 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). Some evidence was available from acute 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-dichloroethylene, 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 weight-of-evidence 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 additivity assumption appears to be suitable in the interest of protecting public health.

The health effects or endpoints of concern for this mixture are hepatic, renal, and developmental effects (all four chemicals), immunological (chloroform, trichloroethylene, vinyl chloride), neurological (chloroform, trichloroethylene), and cancer (chloroform, trichloroethylene, vinyl chloride). To screen this mixture for potential hazards to public health using the 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 by summing the cancer risks for chloroform and vinyl chloride (no unit risk or potency factor is available to estimate risk from trichloroethylene). 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 $1x10^{-4}$, then further evaluation is needed (ATSDR 2004a), using biomedical judgment and community-specific health outcome data, and taking into account community health concerns (ATSDR 1992). If exposures levels are very high (100-fold or more above the MRLs or TTDs), interactions may occur, and their impact on the hazard indexes and cancer risks can be estimated using the weight-of-evidence predictions discussed earlier in this summary.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AKT	α-ketoglutarate transaminase
ALT	alanine aminotransaminase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BINWOE	binary weight-of-evidence
BMDS	Benchmark Dose Software
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
$CHCl_3$	chloroform
$COCl_2$	phosgene
DCE	1,1-dichloroethylene
DCVC	S-(1,2-dichlorovinyl)-L-cysteine
DCVG	S-(1,2-dichlorovinyl)glutathione
DNA	deoxyribonucleic acid
DT	Division of Toxicology
EPA	Environmental Protection Agency
F_1	first-filial generation
GGT	gamma glutamyl transpeptidase
GOT	glutamic oxaloacetic transaminase
GSH	glutathione
HEC	human equivalent concentration
HI	hazard index
IARC	International Agency for Research on Cancer
IgG	immunoglobulin G
Ip	intraperitoneal
IRIS	Integrated Risk Information System
kg	kilogram
L	liter
LDH	lactate dehydrogenase
LEC	lower 95% confidence limit
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
mg	milligram
MRL	Minimal Risk Level
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
ppm	parts per million
PVC	polyvinylchloride
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SDH	sorbitol dehydrogenase
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
TCE	trichloroethylene
TTD	target-organ toxicity dose
TWA	time-weighted average
µg	microgram
U.S.	United States
VC	vinyl chloride
WOE	weight-of-evidence
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to

1. INTRODUCTION

The primary purpose of this Interaction Profile for chloroform, 1,1-dichloroethylene, 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, adequacy of the data as the basis for a mixture Minimal Risk Level (MRL), 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 weight-of-evidence 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 Division of Toxicology and Environmental Medicine's (DTEM) 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.

The chloroform, 1,1-dichloroethylene (DCE), trichloroethylene (TCE), and vinyl chloride (VC) mixture was chosen as the subject for this interaction profile because these chemicals are among the top ten chemicals found in water around hazardous waste sites. They are at the 9th, 7th, 1st, and 8th place, respectively. Consequently, they are also encountered in combinations. All information provided here regarding the occurrence of these chemicals is extracted from the ATSDR's HazDat database (ATSDR 2006) and data are related to completed exposure pathways, i.e., people were/are actually exposed to the chemicals (for definition see ATSDR 1992). For example, the binary combination of 1,1-dichloroethylene and trichloroethylene is the 3rd most often found in contaminated waters and was reported at 62 sites (at 70 sites in all exposure media combined). The binary combination of chloroform and trichloro-

ethylene was found in water at 52 sites which represents the 8th place of occurrence (total 61 sites for all media). Chloroform and 1,1-dichloroethylene were found together at 34 and 28 sites for total media and water, respectively. Trichloroethylene and vinyl chloride combination occurred at 54 sites of which 46 sites had these chemicals together in water. 1,1-Dichloroethylene and vinyl chloride were reported at 25 sites; 23 sites had the chemicals in water media. Finally, the binary combination of chloroform and vinyl chloride was reported at 13 sites in water (19 sites for all media). Exposure to all four chemicals together occurred at 8 sites total and at 6 sites through contaminated water. Previously, ATSDR has developed interaction profiles for other VOCs found frequently in water around hazardous waste sites. These include a mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene and a mixture of benzene, ethylbenzene, toluene, and xylenes (www.atsdr.cdc.gov/interactionprofiles/).

Before evaluating the relevance of joint toxic action data for these chemicals, some understanding of these chemicals and the health endpoints of concern for inhalation and oral exposure is needed. The endpoints of concern include the critical effects that are the bases for 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 2004a).

At room temperature, chloroform is a colorless, volatile liquid with a pleasant, nonirritating odor and a 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 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; effects on the kidney, immune system, nervous system, and the developing organism have also been reported. High-dose chloroform has been used as an anesthetic, but 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 National Toxicology Program's (NTP) Eleventh Report on Carcinogens (NTP 2005) states that chloroform is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. The International Agency for Research on Cancer (IARC 1999a) classifies chloroform as *possibly carcinogenic to humans* (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals. EPA (IRIS 2005) has classified chloroform as *reasonably anticipated to be a human carcinogen* (Group 2B). More information on chloroform is provided in Appendix A and by ATSDR (1997a).

At room temperature, 1,1-dichloroethylene is a colorless, highly volatile liquid with a mild, sweet smell. The primary source of 1,1-dichloroethylene in the environment is industrial production and use (to make polyvinylidene chloride copolymers for plastics, flexible wraps, and flame retardant coatings). 1,1-Dichloroethylene's primary effects following inhalation exposure are on the liver, although effects on the kidney and the developing organism have also been reported. Many of 1,1-dichloroethylene's effects are believed to be the result of metabolism to active products that react with target tissues. The NTP's Eleventh Report on Carcinogens (NTP 2005) does not list 1,1-dichloroethylene. The International Agency for Research on Cancer (IARC) (1999b) notes that 1,1-dichloroethylene is *not classifiable as to its carcinogenicity to humans* (Group 3). EPA (IRIS 2005) has classified 1,1-dichloroethylene as carcinogenicity Group C (*possible human carcinogen*). More information on 1,1-dichloroethylene is provided in Appendix B and by ATSDR (1994).

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 remover, adhesives, and spot removers. Following inhalation or oral exposure, the primary effects of trichloroethylene are neurological (altered visual-motor coordination, drowsiness), with other effects including hepatic, renal, immunological, and developmental also reported. The NTP's Eleventh Report on Carcinogens (NTP 2005) states that trichloroethylene is *reasonably anticipated to be a human carcinogen*. Trichloroethylene, is listed as Group 2A (*possibly carcinogenic to humans*) by IARC, and has not been given a cancer classification by EPA (IRIS 2005). More information on trichloroethylene is provided in Appendix C and by ATSDR (1997b).

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, and for inhalation exposures, renal, immunological, and developmental effects also have been reported. Neurological effects have been reported from very high inhalation exposures. The NTP's Eleventh Report on Carcinogens (NTP 2005) reports that vinyl chloride is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans. IARC (1987) lists vinyl chloride in Group 1 (*carcinogenic to humans*) based on sufficient evidence of carcinogenicity in humans and animals. Vinyl chloride is a *known human carcinogen* (Group A) under the EPA (IRIS 2005) classification scheme. More information vinyl chloride is provided in Appendix D and by ATSDR (2004b).

The critical effects that are the bases for the MRLs, as well as other relatively sensitive effects, are summarized in Table 1. All four chemicals are known to have effects on the liver, kidney, and developing organism. The immunological system is a common target of three of these chemicals, and the nervous system of two. 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. ATSDR toxicological profiles are available for all four of the chemicals that make up the mixture (ATSDR 1994, 1997a, 1997b, 2004b); 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.

Table 1. Potential Health Effects of Concern for Intermediate and Chronic Inhalation and Oral Exposure to the Mixture Chloroform, 1,1-Dichloroethylene, Trichloroethylene, and Vinyl Chloride[a,b]

Endpoint	Chloroform	1,1-Dichloroethylene	Trichloroethylene	Vinyl chloride
Hepatic	**X**	**X**	X	**X**
Renal	X	X	X	X[c]
Immunological	X		X	X[c]
Neurological	X		**X**	
Developmental	X	X	X	X[c]
Cancer	X		X	X

[a]See Appendices A, B, C, and D.
[b]The bases for the MRLs are bolded; other sensitive effects are listed in regular typeface.
[c]Inhalation only

2. JOINT TOXIC ACTION DATA FOR THE MIXTURE OF CONCERN AND COMPONENT MIXTURES

This chapter provides a review and evaluation of the literature pertinent to joint toxic action of the mixture and its components.

2.1 Mixture of Concern

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

2.2 Component Mixtures

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-dichloroethylene, 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 effect), 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-Dichloroethylene

No *in vivo* or *in vitro* studies were located regarding joint toxic actions of chloroform and 1,1-dichloroethylene. No PBPK models specific for co-exposure to chloroform and 1,1-dichloroethylene 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 1994, 1997). At very high co-exposure levels, when the enzyme is saturated, the toxicities of chloroform and 1,1-dichloroethylene 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,-dichloroethylene 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 intraperitoneal administration of chloroform and trichloroethylene on liver endpoints in rats (Anand et al. 2005a). Groups of 3 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 effect addition. Neither the single chemicals nor the binary mixtures resulted in mortality. Plasma alanine aminotransferase (ALT), measured at 24-hour intervals from 24–96 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-effect-additive joint toxic action. In the discussion section of the study publication, the authors state 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 methods and results section of the Anand et al. (2005a) publication, so these statements cannot be evaluated.

Anand et al. (2005a) also invested the effects of joint intraperitoneal administration on the disposition of chloroform and trichloroethylene. Trichloroethylene had no significant effect on blood or liver concentrations of chloroform except for an apparent decrease at the earliest time period of 0.5 hours in blood concentrations of chloroform 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 TCE 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 TCE, 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 intraperitoneal route. Their applicability to other routes of exposure is uncertain.

A similar study by these investigators (Anand et al. 2005b) investigated the joint effects of acute intraperitoneal 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 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 examined the effects of exposure to mixtures of chemicals 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 (Mikiskova and Mikiska 1966), and that blood levels of trichloroethanol correlate better with electrophysiological effects than do 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 3 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 (1 rat), 500 ppm substrate and 500 ppm inhibitor (2 rats), 500 ppm substrate and 10 ppm inhibitor (3 rats) and 500 ppm substrate and 2,000 ppm inhibitor (3 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. 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. This study was concerned only with hepatic effects, and was conducted by intraperitoneal injection in rats. The data are summarized in Table 2, and indicate a less-than-additive interaction. 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 2. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Chloroform and Trichloroethylene

Route, Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no effect	Less than additive		
Ip, 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 = α-ketoglutarate transaminase; $CHCl_3$ = chloroform; Ip = intraperitoneal; 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 1997a, 2004b). 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-Dichloroethylene and Trichloroethylene

Andersen et al. (1987) reported that inhalation co-exposure of groups of 4 male F344 rats to 500 ppm trichloroethylene and a range of concentrations of 1,1-dichloroethylene (~100–1,800 ppm) for a single 6-hour exposure resulted in a protective effect on hepatotoxicity, as assessed by serum GOT (AST) measurement, compared to 1,1-dichloroethylene alone, with higher levels of 1,1-dichloroethylene required to elicit the same serum GOT (AST) increases. The study authors report that similar changes were noted in serum GPT (ALT) levels, but data were not provided in the study. The level of metabolism of 1,1-dichloroethylene 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-dichloroethylene. A protective effect of trichloroethylene on 1,1-dichloroethylene 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-dichloroethylene, but that 50 and 100 ppm trichloroethylene did not have a significant inhibitory effect.

A joint PBPK model for 1,1-dichloroethylene 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. A comparison of model simulations gas uptake data from co-exposure of 1,1-dichloroethylene 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-dichloroethylene 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-dichloroethylene) 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-dichloroethylene and trichloroethylene were located, and those studies evaluated only hepatic endpoints and only to a limited degree. These studies are summarized in Table 3, and indicate a less-than additive effect of trichloroethylene on the hepatotoxicity of 1,1-dichloroethylene, 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 3. Summary of Available Data on the Joint Effects of Simultaneous Exposure to 1,1-Dichloroethylene and Trichloroethylene

Route, Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no effect	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
	(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. 1996b

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

2.2.5 1,1-Dichloroethylene 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-dichloroethylene and vinyl chloride, either together or in succession. 1,1-Dichloroethylene 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 (≈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 authors, vinyl chloride is not known to produce an immediate hepatotoxic response. Simultaneous exposure of fasted male rats to 1000 ppm of vinyl chloride and 200 ppm of 1,1-dichloroethylene (a hepatotoxic concentration) resulted in no changes in serum AKT activity or hepatic histopathology, indicating a protective effect of vinyl chloride on 1,1-dichloroethylene's acute hepatotoxicity. Only when exposure to vinyl chloride was reduced to 201 ppm in another group of rats, exposures to ≈2,000 ppm of 1,1-dichloroethylene 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 changes. 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-dichloroethylene hepatotoxicity due to depletion of GSH by fasting. GSH conjugation detoxifies the reactive metabolites of 1,1-dichloroethylene. 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-dichloroethylene for 4 hours resulted in increased serum AKT and sorbitol dehydrogenase (SDH) levels, whereas exposure of fed rats to 2,000 ppm 1,1-dichloroethylene alone did not increase these indices of liver damage.

No PBPK models specific for co-exposure to 1,1-dichloroethylene 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-dichloroethylene 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-dichloroethylene hepatotoxicity. Results of a sequential exposure experiment were consistent with vinyl chloride depletion of GSH resulting in greater-than-additive hepatotoxicity from dichloroethylene in fed rats (Jaeger et al. 1975). These joint toxic action studies of 1,1-dichloroethylene and vinyl chloride are summarized in Table 4.

Table 4. Summary of Available Data on the Joint Effects of Simultaneous and Sequential Exposure to 1,1-Dichloroethylene and Vinyl Chloride

Route, Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no effect	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 aminotransaminase; DCE = 1,1-dichloroethylene; 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 rats to trichloroethylene and vinyl chloride (up to 5,000 ppm for 6 hours) resulted in decreased depletion of hepatic nonprotein sulfhydryl groups, compared to exposure to vinyl chloride alone. Depletion of nonprotein sulfhydryls (GSH) occurs from conjugation with reactive vinyl chloride metabolites. The effect was observed in rats exposed to 1,000 and 5,000 ppm of vinyl chloride alone, but not to 200 and 600 ppm. At 5,000 ppm exposure, the depletion of GSH was 44%. In contrast, trichloroethylene alone did not appreciably deplete GSH. Results from exposure to the mixture are presented in Table 5.

Table 5. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Trichloroethylene and Vinyl Chloride

Route, Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no effect	Less than additive		
Simultaneous Exposure						
Inhalation, acute	Hepatic/ metabolic (depletion of non-protein sulfhydryls)			VC:TCE % 5,000:600 35 5,000:5,000 20 1,000:1,000 22	Less-than-additive effect of TCE on VC competitive inhibition	Barton et al. 1995

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-dichloroethylene (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 inhalation co-exposure data from gas uptake experiments in groups of 3 male Sprague-Dawley rats assuming competitive, noncompetitive, and uncompetitive inhibition models for shared metabolism by CYP2E1. At concentrations below 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 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.

A joint PBPK model has been described (Barton et al. 1995) that predicts no interaction between the chemicals at levels below 30 ppm of each and a less-than-additive interaction on metabolism at concentrations higher than 30 ppm. Thus, the threshold for metabolic interaction was predicted to be >30 ppm. 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-dichloroethylene, 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, 2004a) to predict the potential impact of interactions. This approach involves determining, for each binary mixture, the weight of evidence 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 6. 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. 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, 2004a). Rationales for the BINWOE

determinations are presented in the tables 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.

Evidence of varying quality and quantity is available supporting projections of joint toxic action for the following pairs of chemicals:

- Chloroform and 1,1-Dichloroethylene
- Chloroform and Trichloroethylene
- Chloroform and Vinyl Chloride
- 1,1-Dichloroethylene and Trichloroethylene
- 1,1-Dichloroethylene 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, 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 6. 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.	*In vivo* data
b.	*In vitro* data
i.	Anticipated route of exposure
ii.	Different route of exposure

Table 7. Effect of Chloroform on 1,1-Dichloroethylene

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

Direction of Interaction – Because both chloroform and 1,1-dichloroethylene 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-dichloroethylene.

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-Dichloroethylene 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-dichloroethylene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels for chloroform and 1,1-dichloroethylene 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-dichloroethylene 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-dichloroethylene 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 8. Effect of 1,1-Dichloroethylene on Chloroform

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

Direction of Interaction – Because both 1,1-dichloroethylene 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-dichloroethylene 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-Dichloroethylene 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-dichloroethylene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels for 1,1-dichloroethylene 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-dichloroethylene 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-dichloroethylene 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 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 high-dose intraperitoneal study. 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 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 therefore could 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-dichloroethylene 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 to the metabolite trichloroethanol (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 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 10. Effect of Trichloroethylene on Chloroform

BINWOE: <IAii for hepatic effects
BINWOE: <IBii for renal effects
BINWOE: <IBii for immunological 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 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 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. 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-dichloroethylene 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 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 11. Effect of Chloroform on Vinyl Chloride

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for immunological 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 therefore, could 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-dichloroethylene 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.

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-di-chloroethylene 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 12. Effect of Vinyl Chloride on Chloroform

BINWOE: <IIBb for hepatic effects
BINWOE: <IIBb for renal effects
BINWOE: <IIBb for immunological 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-dichloroethylene 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-dichloroethylene 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 13. Effect of 1,1-Dichloroethylene 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-dichloroethylene 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-dichloroethylene 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-dichloroethylene 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-dichloroethylene in rats, no toxic effects of trichloroethylene were observed in the studies. No studies were located in which pretreatment with 1,1-dichloroethylene 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 14. Effect of Trichloroethylene on 1,1-Dichloroethylene

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

Direction of Interaction – Because both trichloroethylene and 1,1-dichloroethylene 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-dichloroethylene has been reported in two acute inhalation studies in rats.

Mechanistic Understanding – The effects of 1,1-dichloroethylene 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-dichloroethylene 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 inhalation co-exposure to 500 ppm trichloroethylene and a range of concentrations of 1,1-dichloroethylene (~100–1,800 ppm) resulted in a protective effect on hepatotoxicity compared to 1,1-dichloroethylene alone. El-Masri et al. (1996a) further reported that acute inhalation co-exposure to 500 or 1,000 ppm trichloroethylene inhibited the hepatotoxicity of simultaneous exposure to 1,000 ppm 1,1-dichloroethylene, but that 50 and 100 ppm trichloroethylene did not have a significant inhibitory effect. No studies were located in which pretreatment with trichloroethylene prior to 1,1-dichloroethylene 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 15. Effect of 1,1-Dichloroethylene on Vinyl Chloride

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

Direction of Interaction – Because both 1,1-dichloroethylene 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-dichloroethylene and vinyl chloride

Mechanistic Understanding – 1,1-Dichloroethylene 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 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 two-chemical mixtures that have been evaluated (1,1-dichloroethylene and trichloroethylene, trichloroethylene and vinyl chloride). The exposure levels of 1,1-dichloroethylene 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.

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-dichloroethylene in rats, but no toxic effects of vinyl chloride were reported in the study. No studies were located in which pretreatment with 1,1-dichloroethylene 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-dichloroethylene 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 16. Effect of Vinyl Chloride on 1,1-Dichloroethylene

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

Direction of Interaction – Because both vinyl chloride and 1,1-dichloroethylene 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-dichloroethylene was seen in an acute, high-exposure study in fasted rats.

Mechanistic Understanding – 1,1-Dichloroethylene 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 therefore could 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 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-dichloroethylene, reduced or eliminated the hepatotoxicity of 1,1-dichloroethylene 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-dichloroethylene and tri-chloroethylene, 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-dichloroethylene and vinyl chloride in fasted rats, which are depleted in GSH, resulted in an elimination of the toxicity seen with 1,1-dichloroethylene alone. Studies of longer durations or more environmentally-relevant concentrations were not located. Pre-exposure to a high concentration of vinyl chloride, which depleted GSH, in fed rats resulted in an increased hepatotoxicity of subsequent exposure to 1,1-dichloroethylene (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-dichloroethylene 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 17. Effect of Trichloroethylene on Vinyl Chloride

BINWOE: <IB for hepatic effects
BINWOE: <IB for renal effects
BINWOE: <IB for immunological 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. 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 – Relevant interaction data on pertinent health effects following simultaneous exposure were not located. No studies were located 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-dichloroethylene and trichloroethylene), a rating of "B" was assigned.

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

Table 18. 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. Since a direct demonstration of the mechanism by which the interactions could occur exists, 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 located 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-dichloroethylene 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 of 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 19. 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.

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 chloroform and trichloroethylene, 1,1-dichloroethylene and trichloroethylene, and trichloroethylene and vinyl chloride. 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 WOEs.

Table 19. PBPK models' predictions of interaction thresholds

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

For the individual components, inhalation MRLs are available for all exposure durations for chloroform, for the intermediate duration for 1,1-dichloroethylene, and for acute and intermediate durations for trichloroethylene and vinyl chloride. Oral MRLs are available for all exposure durations for chloroform, for

the chronic duration for 1,1-dichloroethylene, for the acute duration for trichloroethylene, and for the chronic duration for vinyl chloride. All the MRLs for chloroform and the available MRLs for 1,1-dichloroethylene identify hepatic effects as the critical effects. All the available MRLs for trichloroethylene identify neurological effects as the critical effects. The MRLs for intermediate inhalation and chronic oral exposure to vinyl chloride identify hepatic effects as the critical effects; the acute inhalation MRL for vinyl chloride is based on a no-observed-adverse-effect level (NOAEL) for developmental effects (delayed ossification was seen at the LOAEL [lowest-observed-adverse-effect level]), which also was associated with maternotoxicity. The available MRLs are summarized in Table 20.

Table 20. Minimal Risk Levels (MRLs) for the Chemicals of Concern

	Chloroform	1,1-Dichloroethylene	Trichloroethylene	Vinyl chloride
Inhalation				
Acute	0.1 ppm (hepatic effects)	None	2 ppm (neurological effects)	0.5 ppm (developmental effects)
Intermediate	0.05 ppm (hepatic effects)	0.02 ppm (hepatic effects)	0.1 ppm (neurological effects)	0.03 ppm (hepatic effects)
Chronic	0.02 ppm (hepatic effects)	None	None	None
Oral				
Acute	0.3 mg/kg/day (hepatic effects)	None	0.2 mg/kg/day (neurological effects)	None
Intermediate	0.1 mg/kg/day (hepatic effects)	None	None	None
Chronic	0.01 mg/kg/day (hepatic effects)	0.009 mg/kg/day (hepatic effects)	None	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-dichloroethylene, 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.

Because suitable data, joint action models, and PBPK models are lacking for the complete mixture, the recommended approach for the exposure-based assessment of joint toxic action of this mixture for non-cancer endpoints is to use the hazard index method with the TTD modification and qualitative WOE method to assess the potential consequences of 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 equal or exceed 0.1 (Figure 2 of ATSDR 2004a). 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 equals or exceeds 0.1, then no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. As discussed by ATSDR (2004a), 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.

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, 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, 2004a). 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 inhalation exposure are listed in Table 21, which also lists the intermediate inhalation MRLs for each chemical.

Table 21. MRLs and TTDs for Intermediate Inhalation Exposure to Chemicals of Concern[a]

	Chemical			
Endpoint	Chloroform (ppm)	1,1-Dichloroethylene (ppm)	Trichloroethylene (ppm)	Vinyl chloride (ppm)
Hepatic	0.05 (intermediate MRL)	0.02 (intermediate MRL)	1	0.03 (intermediate MRL)
Renal	0.05	0.04	0.7	0.07
Immunological	0.05	Not applicable	0.1	0.03
Neurological	0.05	Not applicable	0.1 (intermediate MRL)	NA
Developmental	0.05	0.05	3	0.5 (acute MRL)

[a]See Appendices A, B, C, and D

With the exception of chloroform, adequate chronic 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 TTDs, and chronic TTD values for chloroform were derived in Appendix A. The chronic inhalation MRL for chloroform is 0.02 ppm and the intermediate inhalation MRL is 0.05 ppm, with both being based on similar physiological effects. 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 data are lacking, the intermediate inhalation TTDs and MRLs for 1,1-dichloroethylene, trichloroethylene, and vinyl chloride be adjusted using a modifying factor of 3 ($10^{0.5}$) when being considered in a chronic exposure scenario. The chronic inhalation TTD values are presented in Table 22, along with the chronic inhalation MRL for chloroform.

Table 22. MRLs and TTDs for Chronic Inhalation Exposure to Chemicals of Concern[a]

Endpoint	Chemical			
	Chloroform (ppm)	1,1-Dichloroethylene (ppm)	Trichloroethylene (ppm)	Vinyl chloride (ppm)
Hepatic	0.02 (chronic MRL)	0.007	0.3	0.01
Renal	0.02	0.04	0.7	0.07
Immunological	0.02	Not applicable	0.03	0.01
Neurological	0.03	Not applicable	0.03	Not applicable
Developmental	0.03	0.02	1	0.2

[a]See 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, 2004a), and are listed, along with MRLs, in Table 23 for intermediate exposure and Table 24 for chronic exposure.

Table 23. MRLs and TTDs for Intermediate Oral Exposure to Chemicals of Concern[a]

Endpoint	Chemical			
	Chloroform (mg/kg/day)	1,1-Dichloroethylene (mg/kg/day)	Trichloroethylene (mg/kg/day)	Vinyl chloride (mg/kg/day)
Hepatic	0.01 (intermediate MRL)	0.3	3	0.003
Renal	0.1	0.3	2	Not applicable
Immunological	0.1	Not applicable	2	Not applicable
Neurological	0.3	Not applicable	0.08	Not applicable
Developmental	0.1	0.3	0.1	Not applicable

[a]See Appendices A, B, C, and D.

Table 24. MRLs and TTDs for Chronic Oral Exposure to Chemicals of Concern[a]

	Chemical			
Endpoint	Chloroform (mg/kg/day)	1,1-Dichloroethylene (mg/kg/day)	Trichloroethylene (mg/kg/day)	Vinyl chloride (mg/kg/day)
Hepatic	0.01 (chronic MRL)	0.009 (chronic MRL)	3	0.003 (chronic MRL)
Renal	0.1	0.009	2	Not applicable
Immunological	0.01	Not applicable	2	Not applicable
Neurological	0.03	Not applicable	0.008	Not applicable
Developmental	0.04	0.009	0.1	Not applicable

[a]See 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 21, 22, 23, and 24, or newer values as they become available. 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_{CHCl3} is the intermediate inhalation exposure to chloroform (in ppm), MRL_{CHCl3} is the intermediate inhalation MRL for chloroform (based on hepatic effects, in ppm), E_{DCE} is the intermediate inhalation exposure to 1,1-dichloroethylene (in ppm), MRL_{DCE} is the intermediate inhalation MRL for 1,1-dichloroethylene (based on hepatic effects, in ppm), E_{TCE} is the intermediate inhalation exposure to tetrachloroethylene (in ppm), $TTD_{TCE,HEPATIC}$ is the intermediate inhalation TTD for hepatic effects of TCE (in ppm), E_{VC} is the intermediate inhalation exposure to vinyl chloride (in ppm), and MRL_{VC} the intermediate inhalation MRL for vinyl chloride (based on hepatic effects, in ppm). The process can be then repeated for each endpoint of concern for intermediate 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 inhalation exposure, using chronic exposure concentrations and chronic inhalation TTDs and MRLs, and for intermediate and chronic oral exposure, for which the exposures are estimated as oral intakes in mg/kg/day, consistent with the units of the intermediate and chronic oral MRLs and TTDs. Components for which data are not available, or which do not affect the endpoint, are not included in the endpoint-specific hazard index calculations.

If the hazard index for effects on an endpoint of concern for any duration and route exceeds one, it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2004a). The impact of interactions from the WOE analysis also is considered. For this particular mixture, the available data on the component pairs support less-than-additive interactions for the individual pairs for most endpoints, as shown in Table 25; for neurological effects of chloroform, the available mechanisms suggest greater-than-additive interactions, and for the neurological effects of trichloroethylene, the direction of interaction is indeterminate. However, since the mechanism behind the interaction is likely to only occur at very high (100-fold or more 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.

If this screening procedure indicates preliminary evidence of a mixture health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2004a). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).

The default 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. 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 equals or exceeds $1x10^{-6}$, then no further assessment of joint toxic action is needed due to the low likelihood that additivity and/or interactions would result in a significant 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 only for chloroform and vinyl chloride, because no unit risk or slope factor is available for tri-chloroethylene. If the sum of the cancer risks for these components for any route and duration of exposure equals or exceeds $1x10^{-4}$, then further evaluation is needed (ATSDR 2004a), using biomedical judgment and community-specific health outcome data, and taking into account community health concerns (ATSDR 1992).

Table 25. Matrix of BINWOE Determinations for Simultaneous Exposure to High Levels of Chemicals of Concern[1]

		ON THE TOXICITY OF			
EFFECT OF		Chloroform	1,1-Dichloroethylene	Trichloroethylene	Vinyl chloride
	Chloroform		<IIBb h,r,d	<IAii h <IBii r,i,d,c ? n	<IIBb h,r,i,d,c
	1,1-Dichloro-ethylene	<IIBb h,r,i,d,c >IIBb n		<IB h,r,i,d,c ? n	<IIBb h,r,i,d,c
	Trichloroethylene	<IAii h <IBii r,i,d,c >IBii n	<IA h <IB r,d		<IB h,r,i,d,c
	Vinyl chloride	<IIBb h,r,i,d,c >IIBb (n)	<IIBb (h,r,d)	<IB h,r,i,d,c ? n	

c = carcinogenic, d = developmental, h = hepatic, i = immunological, n = neurological, r = renal
BINWOE scheme was explained in Table 6. (ATSDR 2001, 2004a)
Some BINWOEs based on results from high level acute exposure studies (see detailes in Tables 7-18)
[1]**Additivity is likely at low level exposures**

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 exceeds one, or the aggregate cancer risks for these chemicals equals or exceeds $1x10^{-4}$, then further evaluation is needed (ATSDR 2004a), using biomedical judgment and community-specific health outcome data, and taking into account 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 weight-of-evidence approach using the BINWOEs summarized in Table 25 could be implemented. These less-than-additive interactions on metabolism, as summarized previously, are likely to only occur at very high (100-fold or more times the corresponding MRL or TTD values) exposure levels. The BINWOEs predict that for toxicities mediated through reactive metabolites (hepatic, renal, immunological, 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 and trichloroethylene), 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).

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-dichloroethylene, trichloroethylene, and vinyl chloride resulting from water contamination near hazardous waste sites. This recommendation is based on the following factors. There are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all four components. Similarly, 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. Finally, available information on toxic actions of the individual components indicates that joint actions of chloroform, 1,1-dichloroethylene, trichloro-ethylene, and vinyl chloride on several toxicity targets are plausible, including hepatic, renal, immunological, 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 was therefore recommended.

Weight-of-evidence 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 as 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, 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 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 equal or exceed 0.1 (ATSDR 2004a). Potential cancer risk is estimated by adding the chemical-specific risks for chloroform 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 exceeds one, or the total cancer risk for these chemicals equals or exceeds $1x10^{-4}$, then further evaluation is needed (ATSDR 2004a), using biomedical judgment and community-specific health

outcome data, and taking into account community health concerns (ATSDR 1992). For very high exposures, 100-fold or more above the MRLs or TTDs, interactions may occur, and their impact can be estimated using the weight-of-evidence results, as summarized above.

5. REFERENCES

Anand SS, Mumtaz MM, Mehendale HM. 2005a. Dose-dependent liver tissue repair after chloroform plus trichloroethylene binary mixture. Basic Clin Pharmacol Toxicol 96(6):436–444.

Anand SS, Mumtaz MM, Mehendale HM. 2005b. Dose-dependent liver regeneration in chloroform, trichloroethylene and allyl alcohol ternary mixture hepatotoxicity in rats. Arch Toxicol 79:671–682.

Andersen ME, Gargas ML, Clewell HJ, et al. 1987. Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene *in vivo* using gas uptake methods. Toxicol Appl Pharmacol 89(2):149–157.

ATSDR. 1992. Public health assessment guidance manual. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service.

ATSDR. 1994. Toxicological profile for 1,1-dichloroethylene. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

ATSDR. 1997a. Toxicological profile for chloroform. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

ATSDR. 1997b. Toxicological profile for trichloroethylene. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

ATSDR. 2001. Guidance manual for the preparation of an interaction profile. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR 2004a. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2004b. Toxicological profile for vinyl chloride. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

Barton HA, Creech JR, Godin CS, et al. 1995. Chloroethylene mixtures: pharmacokinetic modeling and *in vitro* metabolism of vinyl chloride, trichloroethylene, and trans-1,2-dichloroethylene in rat. Toxicol Appl Pharmacol 130(2):237–247.

Blain L, Lachapelle P, Molotchnikoff. 1992. Evoked potentials are modified by long term exposure to trichloroethylene. Neurotoxicology 13:203–206. (As cited in ATSDR 1997 and PubMed.)

Blain L, Lachapelle P, Molotchnikoff. 1994. Electroretinal responses are modified by chronic exposure to trichloroethylene. Neurotoxicology 15:627–631. (As cited in ATSDR 1997 and PubMed.)

Constan AA, Yang RS, Baker DC, Benjamin SA. 1995. A unique pattern of hepatocyte proliferation in F344 rats following long-term exposures to low levels of a chemical mixture of groundwater contaminants. Carcinogenesis 16(2):303–310.

El-Masri HA, Constan AA, Ramsdell HS, et al. 1996a. Physiologically based pharmacodynamic modeling of an interaction threshold between trichloroethylene and 1,1-dichloroethylene in Fischer 344 rats. Toxicol Appl Pharmacol 141(1):124–132.

El-Masri HA, Tessari JD, Yang RSH. 1996b. Exploration of an interaction threshold for the joint toxicity of trichloroethylene and 1,1-dichloroethlene: utilization of a PBPK model. Arch Toxicol 70(9):527–539.

IARC. 1987. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7.

IARC. 1999a. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 73. Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances.

IARC. 1999b. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. Re-Evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide.

IRIS. 2005. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. http://www.epa.gov/iris/. October 14, 2005.

Isaacs KK, Evans MV, Harris TR. 2004. Visualization-based analysis for a mixed-inhibition binary PBPK model: determination of inhibition mechanism. J Pharmacokinet Pharmacodyn 31(3):215–242.

Jaeger RJ, Conolly RB, Reynolds ES, and Murphy SD. 1975. Biochemical toxicology of unsaturated halogenated monomers. Environ Health Perspect 11:121–128.

Mikiskova H, Mikiska A. 1966. Trichloroethanol in trichloroethylene poisoning. Br J Ind Med 23:116–125.

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. October 14, 2005.

APPENDIX A: BACKGROUND INFORMATION FOR CHLOROFORM

This appendix was written based primarily on the Toxicological Profile for Chloroform (ATSDR 1997). 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 Toxicokinetics

Following inhalation exposure, absorption of chloroform appears to be rapid and extensive. While numerous studies in humans have demonstrated that inhaled chloroform is absorbed into the blood and extrarespiratory tissues, a quantitative measurement of absorption fraction or rate has not been reported (Aggazzotti et al. 1993; Cammann and Hübner 1995; Levesque et al. 1994; Nashelsky et al. 1995). Animal toxicity studies of inhaled chloroform have provided evidence for absorption, but quantitative estimates have not been reported (see ATSDR 1997). A study of absorption of an oral dose of ^{13}C-labeled chloroform (0.5 grams 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 ^{14}C-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 by both routes.

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 ^{14}C-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 following inhalation exposure, with the primary concentrations in lipophilic tissues (Brown et al. 1974; Pfaffenberger et al. 1980; Taylor et al. 1974).

Metabolism of chloroform occurs primarily by cytochrome p-450-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 ($V_{max}C$ = 15.7 mg/hour/kg, Km = 0.448 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-dependant. 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 1997), 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; Gearhart et al. 1993; McKone 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 (Lunt 1953; Royston 1924; Smith et al. 1973; Townsend 1939; Whitaker and Jones 1965). 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 glutamic pyruvic transaminase [SGPT] and serum glutamic oxaloacetic transaminase [SGOT] 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 (Baeder and Hofmann 1988; Culliford and Hewitt 1957; Deringer et al. 1953; Ikatsu and Nakajima 1992; Kylin et al. 1963; Lundberg et al. 1986; Schwetz et al. 1974; Torkelson et al. 1976).

The liver is a primary target of oral chloroform toxicity in humans, with some evidence that suggests that 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 SGOT, SGPT, and lactate dehydrogenase (LDH) activities and increased bilirubin levels). At autopsy, fatty degeneration and extensive centrilobular necrosis were observed in one fatal case (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 oral doses of 34 mg/kg or greater, hepatic effects included increased liver weight, fatty changes, and necrosis (Jones et al. 1958; Larson et al. 1994b; Moore et al. 1982; Munson et al. 1982; Nakajima et al. 1995; Ruddick et al. 1983; Thompson et al. 1974; Wang et al. 1994, 1995). A NOAEL of 26 mg/kg/day (4 days) was identified in mice (Larson et al. 1994b). 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. 1995b). 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 (Bull et al. 1986; Chu et al. 1982a, 1982b; Eschenbrenner and Miller 1945; Larson et al. 1995a, 1995b; Munson et al. 1982; Palmer et al. 1979; Pereira 1994). 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 the dog (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 1976; Tumasonis et al. 1985, 1987); the lowest level at which chronic effects were seen was 15 mg/kg/day, the lowest exposure tested, in the dog (Heywood et al. 1979).

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 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 (Culliford and Hewitt 1957; Deringer et al. 1953; Larson et al. 1996; Torkelson et al. 1976). Acute, high-dose oral exposure to chloroform in humans results in albinuria, 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, high-dose animal studies of oral chloroform exposure have also identified renal effects, including cytoplasmic vacuolization, swelling, and necrosis of proximal tubule cells (Chu et al. 1982a; Larson et al. 1993, 1995a, 1995b; Moore et al. 1982; Thompson et al. 1974). Intermediate-duration animal studies have also identified renal changes, including increased kidney weight, inflammation, renal cell proliferation, and proximal tubular necrosis (Chu et al. 1982a; Gulati et al. 1988; EPA 1980; Larson et al. 1994a, 1994b, 1995a, 1995b; Lipsky et al. 1993; Munson et al. 1982; Palmer et al. 1979). The lowest LOAEL and NOAEL reported by ATSDR (1997) for renal effects of intermediate duration oral exposure are 6.0 and 17.4 mg/kg/day for increased foci of regenerating renal proximal tubules in mice given chloroform in their drinking water for 3 weeks (Larson et al. 1995a). In chronic oral studies, no definite renal effects were observed in rats exposed to ≤200 mg/kg/day or mice exposed to <477 mg/kg/day time-weighted average (TWA) (Heindel et al. 1995; Jorgenson et al. 1985; NCI 1976;

Roe et al. 1979). In dogs, however, 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).

Immunological Effects: Some evidence of immunological effects from inhalation exposure to chloroform has been reported in humans, for which a LOAEL of 2 ppm for splenomegaly was identified in humans exposed occupationally for 1–4 years (Bomski et al. 1967). A 6-month inhalation study in animals did not detect splenic changes in rats exposed to 25 ppm of chloroform (Torkelson et al. 1976). Information on potential immunological effects in humans exposed orally to chloroform was not located. Acute and intermediate duration oral studies have identified reduced lymphocyte counts in rats (Chu et al. 1982a), and depression of humoral immunity (assessed as antibody-forming cells/spleen) and at higher doses, cell-mediated immunity (delayed hypersensitivity) in mice (Munson et al. 1982). A LOAEL of 50 mg/kg/day of chloroform for depression of humoral immunity was identified in mice treated for 14 and for 90 days, with effects being more marked at the shorter duration (Munson et al. 1982).

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 ≈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). Exhaustion was reported in 10 women exposed to ≥22 ppm chloroform during intermediate- and chronic-duration occupational exposures (Challen et al. 1958). Chronic exposure to chloroform concentrations ≥77 ppm caused exhaustion, lack of concentration, depression, or irritability in 9 of 10 occupationally exposed women. A case report of an individual addicted to chloroform inhalation for ≈12 years reported psychotic episodes, hallucinations and delusions, and convulsions (Heilbrunn et al. 1945). Neurological effects have been reported in case reports of humans who ingested very high doses of chloroform (Piersol et al. 1933; Schroeder 1965; Storms 1973) and in oral studies in animals (Balster and Borzelleca 1982; Bowman et al. 1978; Jones et al. 1958; Kanada et al. 1994; Landauer et al. 1982), with overt signs generally seen only at very high exposure levels. The NOAEL and LOAEL for neurobehavioral effects were 31.1 mg/kg/day (up to 90 days) and 100 mg/kg/day (60 days), 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 not available, and the single study of chloroform-associated developmental effects following exposure to chloroform through disinfected drinking water is confounded by co-exposure to numerous

other substances (Kramer et al. 1992). Animal studies of chloroform inhalation have consistently identified developmental effects, including growth retardation, decreased crown-rump length, altered ossification, cleft palate, and fetal resorption (Baeder and Hofmann 1988; Murray et al. 1979; Newell and Dilley 1978; Schwetz et al. 1974), generally beginning at 30 ppm chloroform or greater. Oral exposure of rats to 316 mg/kg/day or greater on days 6–15 of gestation has 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 for decreased fetal weight were 50 and 126 mg/kg/day on gestation days 6–15 in the rat (Thompson et al. 1974). A serious LOAEL of 63 mg/kg/day on days 6–18 of gestation for abortion in rabbits was reported by ATSDR (1997), but this LOAEL is not well supported because it was from the preliminary range-finding portion of a study with only 5 rabbits/group, with no report of results in controls, and in which abortion occurred in both controls and treated animals in the main part of the study (Thompson et al. 1974). The potential developmental toxicity of intermediate oral exposure to chloroform is even less well characterized in animals. No neurobehavioural 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, F_1 males had increased epidiymal weights and degeneration of the epidiymal epithelium and F_1 females had increased liver weight and hepatocellular degeneration at 41 mg/kg/day for 105 days (Gulati et al. 1988).

Cancer: No studies were located regarding cancer in humans or animals after inhalation exposure to chloroform. Epidemiology studies suggest an association between cancer in humans and the consumption of chlorinated drinking water, but the results are not conclusive at this time (Alavanja et al. 1978; Cantor et al. 1978; Ijsselmuiden et al. 1992; McGeehin et al. 1993; Young et al. 1981; Zierler et al. 1988). 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. Evidence of chloroform carcinogenicity is mixed following intermediate-duration oral exposure in animals, with studies suggesting that following exposures of <52 weeks to <250 mg/kg/day, no increase in tumor formation is noted (Klaunig et al. 1986; Stoner et al. 1986) but with one study reporting 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 animal studies, resulting in tumors of the liver and kidney (Dunnick and Melnick 1993; Jorgenson et al. 1985; NCI 1976; Roe et al. 1979; Tumasonis et al. 1987). In general, studies of exposure levels of 60 mg/kg/day or greater 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 (primary) and kidneys (secondary) 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). 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).

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 central nervous system depression, and are 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 (SGPT, SGOT, 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 (1997) derived an acute-duration inhalation MRL of 0.1 ppm for chloroform, based on a NOAEL of 3 ppm for hepatic changes in mice exposed for 7 days and an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

ATSDR (1997) derived an intermediate-duration inhalation MRL of 0.05 ppm for chloroform, based on a LOAEL of 14 ppm in human workers for vomiting and toxic hepatitis. The LOAEL of 14 ppm was divided by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) and a modifying factor of 3 (insufficient diagnostic data to determine the seriousness of hepatotoxic effects) to arrive at the MRL of 0.05 ppm.

ATSDR (1997) derived a chronic-duration inhalation MRL of 0.02 ppm for chloroform, based on a LOAEL of 2 ppm for hepatic effects (hepatomegaly, fatty liver, jaundice) in chloroform-exposed workers. The LOAEL of 2 ppm was divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability) to arrive at the MRL of 0.02 ppm.

ATSDR (1997) 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 in mice (Larson et al. 1994b) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR (1997) derived an intermediate-duration oral MRL of 0.1 mg/kg/day for chloroform, based on a NOAEL of 15 mg/kg/day for hepatic effects 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 (1997) derived a chronic-duration oral MRL of 0.01 mg/kg/day for chloroform, based on a LOAEL of 15 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 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

EPA (IRIS 2005) has not derived a reference concentration (RfC) for chloroform.

EPA (IRIS 2005) derived a reference dose (RfD) of 0.01 mg/kg/day for chloroform, based on a LOAEL of 15 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 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Under the 1986 U.S. EPA Guidelines for Carcinogen Risk Assessment, chloroform has been classified as Group B2, *probable human carcinogen*, based on "sufficient evidence" of carcinogenicity in animals (IRIS 2005). Under U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (EPA 1996), 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 (IRIS 2005).

Chloroform is *not likely to be carcinogenic to humans* by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration. Due to the mode of action of chloroform carcinogenicity (repeated cellular damage and regenerative hyperplasia), the RfD of 0.01 mg/kg/day can be considered protective against cancer risk for chloroform.

NTP's Eleventh Report on Carcinogens (NTP 2005) states that chloroform is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. 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 (TTD) Values

The endpoints of concern for chloroform in this mixture are hepatic, renal, immunological, neurological, and developmental. For endpoints that are not the basis of the MRL, TTDS are derived below, using the methods described by ATSDR (2004). The derivations are based primarily on data provided in ATSDR (1997), and in particular, the Levels of Significant Exposure (LSE) tables.

Inhalation TTDs

Following EPA (1994) methodology, the human equivalent concentration ($NOAEL_{HEC}$) for an extrarespiratory effect produced by a category 3 gas, such as chloroform, is calculated by multiplying the duration-adjusted animal NOAEL by the ratio of the blood:gas partition coefficients in animals and humans [(Hb/g)A / Hb/g)H]. Since the partition coefficients in rodents are greater than in humans (see ATSDR 1997), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation: The intermediate inhalation MRL for chloroform is 0.05 ppm based on hepatic effects.

Renal Effects, Intermediate Inhalation: Larson et al. (1996) identified a NOAEL of 1.99 ppm and a LOAEL of 10 ppm for nephropathy and enlarged nuclei of the proximal tubule cells of male mice exposed to chloroform for 6 hours/day, 7 days/week, for 13 weeks. The NOAEL of 1.99 ppm was duration-adjusted to 0.5 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 0.5 ppm as described in the Toxicological Profile. Application of an uncertainty factor of 30 (3 for animal to human extrapolations and 10 for intrahuman variability) would yield a TTD_{RENAL} of 0.02 ppm. However, as this would fall below the MRL, the intermediate-duration MRL of 0.05 ppm will be adopted as the TTD_{RENAL} for chloroform.

Immunological Effects, Intermediate Inhalation: The Toxicological Profile for Chloroform (ATSDR 1997) lists only one intermediate-duration inhalation study that evaluated immunological effects of chloroform (Torkelson et al. 1976). However, the study did not identify an effect level for immunological effects, making it unsuitable for use in TTD derivation. The chronic study of Bomski et al. (1967) identified immunological effects as sensitive effects in humans following chronic exposure, resulting in a chronic TTD_{IMMUNO} equal to the chronic MRL. The intermediate TTD_{IMMUNO} is therefore set at 0.05 ppm, equal to the intermediate MRL.

Neurological Effects, Intermediate Inhalation: Adequate studies of the neurological effects of chloroform following intermediate-duration inhalation exposure are not available. Chronic exposure to chloroform has resulted in neurological effects, including dizziness, fatigue, somnolence, insomnia, and anorexia, in workers exposed to 13.49 ppm chloroform for 1–15 years (Li et al. 1993). The chronic TTD_{NEURO}, based on these effects, is 0.03 ppm. As this is below the intermediate-duration MRL and no intermediate-duration studies are available to derive an intermediate-duration TTD_{NEURO}, the intermediate-duration MRL of 0.05 ppm will be adopted as the TTD_{NEURO}.

Developmental Effects, Intermediate Inhalation: Both Schwetz et al. (1974) and Baeder and Hoffman (1988) reported less serious developmental LOAELs of 30 ppm in rats exposed for 7 hours/day during organogenesis. The LOAEL was adjusted to 8.75 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 8.75 ppm as described previouly 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 intrahuman variability) would yield a TTD_{DEVEL} of 0.03 ppm. However, as this would fall below the MRL, the intermediate-duration MRL of 0.05 ppm will be adopted as the TTD_{RENAL} for chloroform.

Hepatic Effects, Chronic Inhalation: The chronic inhalation MRL for chloroform is 0.02 ppm based on hepatic effects.

Renal Effects, Chronic Inhalation: Larson et al. (1996) identified a NOAEL of 1.99 ppm and a LOAEL of 10 ppm for nephropathy and enlarged nuclei of the proximal tubule cells of male mice exposed to chloroform for 6 hours/day, 7 days/week, for 13 weeks. The NOAEL of 1.99 ppm was duration-adjusted to 0.5 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 0.5 ppm as described previously under the the heading Inhalation TTDs. Application of an uncertainty factor of 30 (3 for animal to human extrapolations using a dosimetric adjustment) and 10 for intrahuman variability) yields a TTD_{RENAL} of 0.02 ppm.

Immunological Effects, Chronic Inhalation: In the same study from which the MRL was derived for hepatic effects, splenomegaly was reported at the same exposure level, 2 ppm, as hepatic effects in humans exposed to chloroform by inhalation for 1–4 years (Bomski et al. 1967). The MRL of 0.02 ppm is therefore applicable for immunological effects as well.

Neurological Effects, Chronic Inhalation: Li et al. (1993) reported numerous neurological effects, including dizziness, fatigue, somnolence, insomnia, and anorexia, in workers exposed to 13.49 ppm chloroform for 1–15 years. The LOAEL of 13.49 ppm was duration-adjusted for a continuous exposure scenario, resulting in a $LOAEL_{HEC}$ of 3.21 ppm. An uncertainty factor of 100 (10 for use of a LOAEL and 10 for intrahuman variability) was applied to derive the TTD_{NEURO} of 0.03 ppm.

Developmental Effects, Chronic Inhalation: Both Schwetz et al. (1974) and Baeder and Hofmann (1988) reported less serious developmental LOAELs of 30 ppm in rats exposed for 7 hours/day during organogenesis. The LOAEL was adjusted to 8.75 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 8.75 ppm 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 intrahuman variability) yields a TTD_{DEVEL} of 0.03 ppm.

Oral TTDs

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

Renal Effects, Intermediate Oral: Larson et al. (1995a) identified a NOAEL of 6.0 mg/kg/day and a LOAEL of 17.4 mg/kg/day for increased foci of regenerating renal proximal tubules in mice given chloroform in their drinking water for 3 weeks (Larson et al. 1995a). Application of an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for intrahuman variability) would result in a TTD_{RENAL} of 0.06 mg/kg/day. Because this value is lower than the MRL, the intermediate-duration oral MRL of 0.1 mg/kg/day will be adopted as the TTD_{RENAL} for chloroform

Immunological Effects, Intermediate Oral: Munson et al. (1982) identified a LOAEL for depressed humoral immunity in mice dosed orally with 50 mg/kg/day of chloroform for 90 days. No NOAEL was identified. Because application of an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) to this LOAEL would result in a TTD_{IMMUNO} that is less than the MRL, the intermediate oral MRL of 0.1 mg/kg/day is adopted as the TTD_{IMMUNO}

Neurological Effects, Intermediate Oral: The NOAEL and LOAEL for neurobehavioral effects were 31.1 mg/kg/day (up to 90 days) and 100 mg/kg/day (60 days), as determined by a battery of behavioral tests in mice administered chloroform by gavage in aqueous emulphor (Balster and Borzelleca 1982). Application of an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for intrahuman variability) to the NOAEL of 15 mg/kg/day 6 days/week (12.9 mg/kg/day for continuous exposure) results in a TTD_{NEURO} of 0.3 mg/kg/day.

Developmental Effects, Intermediate Oral: Gulati et al. (1988) reported that, in a continuous breeding study in mice, F_1 males had increased epidiymal weights and degeneration of the epidiymal epithelium and F_1 females had increased liver weight and hepatocellular degeneration following oral dosing (starting with the parental generation) with 41 mg/kg/day for 105 days (Gulati et al. 1988). Application of an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) would result in a TTD_{DEVEL} of 0.04, which is lower than the MRL. Therefore, the intermediate-duration oral MRL of 0.1 mg/kg/day will be adopted as the TTD_{DEVEL} for chloroform.

Hepatic Effects, Chronic Oral: The chronic oral MRL for chloroform is 0.01 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 (Heywood et al. 1979). An uncertainty factor of 100 (10 for animal to human extrapolations and 10 for intrahuman variability) is applied to the NOAEL of 15 mg/kg/day 6 days/week (12.9 mg/kg/day for continuous exposure), resulting in a TTD_{RENAL} of 0.1 mg/kg/day for chronic oral exposure.

Immunological Effects, Chronic Oral: Data for chronic exposure were not available. An intermediate duration oral study indicates that immunological effects, although not supported by a large database, may be sensitive effects of oral exposure to chloroform, and is supported by some data for the inhalation route. Therefore, it is recommended that the chronic oral MRL of 0.01 mg/kg/day be adopted as the TTD_{IMMUNO} for chloroform.

Neurological Effects, Chronic Oral: No chronic oral study of sensitive endpoints for neurological effects was available for chloroform. The NOAEL for neurobehavioral effects from intermediate exposure was 31.1 mg/kg/day in mice administered chloroform by gavage in aqueous emulphor for durations up to 90 days (Balster and Borzelleca 1982). In the same study, neurobehavioral effects were not seen at 100 mg/kg/day for 30 days, but did occur at this dose level after 60 days of exposure.

Application of an uncertainty factor of 100 (10 for animal to human extrapolations, 10 for intrahuman variability, and 10 for extrapolation from intermediate to chronic duration) to the NOAEL results in a TTD_{NEURO} of 0.03 mg/kg/day. The duration uncertainty factor was considered necessary because extending the duration of exposure in the intermediate duration study resulted in the expression of effects, and 10 was chosen because the chronic oral MRL is 10-fold lower than the intermediate oral MRL for chloroform.

Developmental Effects, Chronic Oral: Gulati et al. (1988) reported that, in a continuous breeding study in mice, F_1 males had increased epidiymal weights and degeneration of the epidiymal epithelium and F_1 females had increased liver weight and hepatocellular degeneration following oral dosing (starting with the parental generation) with 41 mg/kg/day (Gulati et al. 1988). Application of an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal to human extrapolation, and 10 for intrahuman variability) results in a TTD_{DEVEL} of 0.04 mg/kg/day.

Summary (TTD for Chloroform)

Intermediate Inhalation TTDs:

$MRL_{HEPATIC}$ = 0.05 ppm
TTD_{RENAL} = 0.05 ppm
TTD_{IMMUNO} = 0.05 ppm
TTD_{NEURO} = 0.05 ppm
TTD_{DEVEL} = 0.05 ppm

Chronic Inhalation TTDs:

$MRL_{HEPATIC}$ = 0.02 ppm
TTD_{RENAL} = 0.02 ppm
TTD_{IMMUNO} = 0.02 ppm
TTD_{NEURO} = 0.03 ppm
TTD_{DEVEL} = 0.03 ppm

Intermediate Oral TTDs:

$MRL_{HEPATIC}$ = 0.1 mg/kg/day
TTD_{RENAL} = 0.1 mg/kg/day
TTD_{IMMUNO} = 0.1 mg/kg/day
TTD_{NEURO} = 0.3 mg/kg/day
TTD_{DEVEL} = 0.1 mg/kg/day

Chronic Oral TTDs:

$MRL_{HEPATIC}$ = 0.01 mg/kg/day

TTD_{RENAL} = 0.1 mg/kg/day
TTD_{IMMUNO} = 0.01 mg/kg/day
TTD_{NEURO} = 0.03 mg/kg/day
TTD_{DEVEL} = 0.04 mg/kg/day

A.6 References

Ade P, Guastadisegni C, Testai E, et al. 1994. Multiple activation of chloroform in kidney microsome from male and female DBA/2J mice. J Biochem Toxicol 9(6):289–295.

Aggazzotti G, Fantuzzi G, Righi E, et al. 1993. Chloroform in alveolar air of individuals attending indoor swimming pools. Arch Environ Health 48(4):250–254.

Aiking H, Van Acker MB, Scholten RJPM, et al. 1994. Swimming pool chlorination: a health hazard? Toxicol Lett 72 (l-3):375–380.

Alavanja M, Goldstein I, Susser M. 1978. A case control study of gastrointestinal and urinary tract cancer mortality and drinking water chlorination. In: Jolley RJ, Gorchen H, Hamilton DH Jr., eds. Water chlorination, environmental impact and health effects. Ann Arbor, MI: Ann Arbor Science Publications, 1:395–409.

ATSDR. 1997. Toxicological profile for chloroform. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

ATSDR 2004. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Baeder C, Hofmann T. 1988. Inhalation embryotoxicity study of chloroform in Wistar rats. Frankfurt: Pharma Research Toxicology and Pathology, Hoechst Aktiengesellschaft.

Balster RL, Borzelleca JF. 1982. Behavioral toxicity of trihalomethane contaminants of drinking water in mice. Environ Health Perspect 46:127–136.

Bomski H, Sobolewska A, Strakowski A. 1967. [Toxic damage of the liver by chloroform in chemical industry workers.] Int Arch F Gewerbepathologie u. Gewerbehygiene 24:127–134. (German)

Bowman FJ, Borzelleca JF, Munson AE. 1978. The toxicity of some halomethanes in mice. Toxicol Appl Pharmacol 44:213–215.

Branchflower RV, Nunn DS, Highet RJ, et al. 1984. Nephrotoxicity of chloroform: metabolism to phosgene by the mouse kidney. Toxicol Appl Pharmacol 72:159–168.

Brown DM, Langley PF, Smith D, et al. 1974. Metabolism of chloroform. I. The metabolism of ^{14}C-chloroform by different species. Xenobiotica 4:151–163.

Bull RJ, Brown JM, Meierhenry EA. 1986. Enhancement of the hepatotoxicity of chloroform in B6C3Fl mice by corn oil: implications for chloroform carcinogenesis. Environ Health Perspect 69:49–58.

Burkhalter JE, Balster RL. 1979. Behavioral teratology evaluation of trichloromethane in mice. Neurobehav Toxicol 1:199–205.

Cammann K, Hübner K. 1995. Trihalomethane concentrations in swimmers' and bath attendants' blood and urine after swimming or working in indoor swimming pools. Arch Environ Health 50(1):61–65.

Cantor KP, Hoover R, Mason TJ, et al. 1978. Associations of cancer mortality with halomethanes in drinking water. J Natl Cancer Inst 61:979–985.

Challen PJR, Hickish DE, Bedford J. 1958. Chronic chloroform intoxication. Br J Ind Med 15:243–249.

Chinery RL, Gleason AK. 1993. A compartmental model for the prediction of breath concentration and absorbed dose of chloroform after exposure while showering. Risk Anal 13 (1):5l–62.

Chu I, Villeneuve DC, Secours VE, et al. 1982a. Toxicity of trihalomethanes: I. The acute and subacute toxicity of chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J Environ Sci Health B 17:205–224.

Chu I, Villeneuve DC, Secours VE, et al. 1982b. Trihalomethanes: II. Reversibility of toxicological changes produced by chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J Environ Sci Health B 17:225–240.

Cohen EN, Hood N. 1969. Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. Anesthesiology 30:306–314.

Corley RA, Mendrala AL, Smith FA, et al. 1990. Development of a physiologically based pharmacokinetic model for chloroform. Toxicol Appl Pharmacol 103:5 12–527.

Culliford D, Hewitt HB. 1957. The influence of sex hormone status on the susceptibility of mice to chloroform-induced necrosis of the renal tubules. J Endocrinol 14:381–393.

Danielsson BRG, Ghantous H, Dencker L. 1986. Distribution of chloroform and methyl chloroform and their metabolites in pregnant mice. Biol Res Pregnancy 7:77–83.

Deringer MK, Dunn TB, Heston WE. 1953. Results of exposure of strain C3H mice to chloroform. Proc Soc Exp Biol Med 83:474–479.

De Salva S, Volpe A, Leigh G, et al. 1975. Long-term safety studies of a chloroform-containing dentifrice and mouth-rinse in man. Food Cosmet Toxicol 13:529–532.

Docks EL, Krishna G. 1976. The role of glutathione in chloroform-induced hepatotoxicity. Exp Mol Pathol 24:13–22.

Dunnick JK, Melnick RL. 1993. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. J Natl Cancer Inst 85(10):817–822.

EPA. 1980. Effects of chloroform in the drinking water of rats and mice: ninety-day subacute toxicity study. Cincinnati, OH: U.S. Environmental Protection Agency, Health Effects Research Laboratory, Office of Research and Development. PB80219108. EPA600180030.

EPA. 1986. Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. Federal Register 51(185):33992–34003.

EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency. EPA600890066F.

EPA. 1996. Proposed guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. Federal Register 61(79):17960–18011.

Eschenbrenner AB, Miller E. 1945. Induction of hepatomas in mice by repeated oral administration of chloroform, with observations on sex differences. J Natl Cancer Inst 5:251–255.

Featherstone HW. 1947. Chloroform. Anesthesiology 8:362–371.

Fry BJ, Taylor R, Hathaway DE. 1972. Pulmonary elimination of chloroform and its metabolite in man. Arch Int Pharmacodyn 196:98–111.

Gearhart JM, Seckel C, Vinegar A. 1993. *In vivo* metabolism of chloroform in B6C3Fl mice determined by the method of gas uptake: the effects of body temperature on tissue partition coefficients and metabolism. Toxicol Appl Pharmacol 119(2):258–266.

Gettler AO, Blume H. 1931. Chloroform in the brain, lungs, and liver. Quantitative recovery and determination. Arch Pathol 11:554–560.

Goodman LS, Gilman A. 1980. The pharmacological basis of therapeutics. 6th ed. New York, NY: MacMillan Publishing.

Gordon SM, Wallace LA, Pellizzari ED, et al. 1988. Human breath measurements in a clean-air chamber to determine half-lives for volatile organic compounds. Atmos Environ 22:2165–2170.

Gulati DK, Hope E, Mounce RC, et al. 1988. Chloroform: reproduction and fertility assessment in CD-l mice when administered by gavage. Research Triangle Park, NC: National Toxicology Program, National Institute of Environmental Health Sciences.

Heilbrunn G, Liebert E, Szanto PB. 1945. Chronic chloroform poisoning: clinical and pathologic report of a case. Arch Neurol Psych 53:68–72.

Heindel JJ, Chapin RE, George J, et al. 1995. Assessment of the reproductive toxicity of a complex mixture of 25 groundwater contaminants in mice and rats. Fundam Appl Toxicol 25:9–19

Heywood R, Sortwell RJ, Noel PRB, et al. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835–851.

IARC. 1999. Chloroform. International Agency for Research on Cancer. IARC Monographs Volume 73, 131.

Ijsselmuiden CB, Gaydos C, Feighner B, et al. 1992. Cancer of the pancreas and drinking water: a population based case control study in Washington County, Maryland. Am J Epidemiol 136(7):836–842.

Ikatsu H, Nakajima T. 1992. Hepatotoxic interaction between carbon tetrachloride and chloroform in ethanol treated rats. Arch Toxicol 66(8):580–586.

IRIS. 2005. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. http://www.epa.gov/iris/. October 14, 2005.

Jones WM, Margolis G, Stephen CR. 1958. Hepatotoxicity of inhalation anesthetic drugs. Anesthesiology 19:715–723.

Jorgenson TA, Meierhenry EF, Rushbrook CJ, et al. 1985. Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fundam Appl Toxicol 5:760–769.

Kanada M, Miyagawa M, Sato M, et al. 1994. Neurochemical profile of effects of 28 neurotoxic chemicals on the central nervous system in rats (1) effects of oral administration on brain contents of biogenic amines and metabolites. Ind Health 32:145–164.

Klaunig JE, Ruth RJ, Pereira MA. 1986. Carcinogenicity of chlorinated methane and ethane compounds administered in drinking water to mice. Environ Health Perspect 69:89–95.

Kramer MD, Lynch CF, Isacson P, et al. 1992. The association of waterborne chloroform with intrauterine growth retardation. Epidemiology 3(5):407–413.

Kylin B, Reichard H, Sumegi I, et al. 1963. Hepatotoxicity of inhaled trichloroethylene, tetrachloroethylene and chloroform. Single exposure. Acta Pharmacol Toxicol 20:16–26.

Landauer MR, Lynch MR, Balster RL, et al. 1982. Trichloromethane-induced taste aversions in mice. Neurobehav Toxicol Teratol 4:305–309.

Larson JL, Sprankle CS, Butterworth BE. 1994a. Lack of chloroform-induced DNA repair *in vitro* and *in vivo* in hepatocytes of female B6C3F1 mice. Environ Mol Mutagen 23(2):132–136.

Larson JL, Templin MV, Wolf DC. 1996. A 90-day chloroform inhalation study in female and male B6C3Fl mice: implications for cancer risk assessment. Fundam Appl Toxicol 30:118–137.

Larson JL, Wolf DC, Butterworth BE. 1993. Acute hepatotoxic and nephrotoxic effects of chloroform in male F-344 rats and female B6C3Fl mice. Fundam Appl Toxicol 20(3):302–315.

Larson JL, Wolf DC, Butterworth BE. 1994b. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs. *ad libitum* in drinking water. Fundam Appl Toxicol 22:90–102.

Larson JL, Wolf DC, Butterworth BE. 1995a. Induced regenerative cell proliferation in livers and kidneys of male F-344 rats given chloroform in corn oil by gavage or *ad libitum* in drinking water. Toxicology 95:73–86.

Larson JL, Wolf DC, Mery S, et al. 1995b. Toxicity and cell proliferation in the liver kidneys and nasal passages of female F-344 rats, induced by chloroform administered by gavage. Food Chem Toxicol 33(6):443–456.

Levesque B, Ayotte P, LeBlanc A, et al. 1994. Evaluation of dermal and respiratory chloroform exposure in humans. Environ Health Perspect 102(12):1082–1087.

Li LH, Jiang XZ, Liang YX, et al. 1993. Studies on the toxicity and maximum allowable concentration of chloroform. Biomed Environ Sci 6(2):179–186.

Lipsky MM, Skinner M, O'Connell C. 1993. Effects of chloroform and bromodichloromethane on DNA synthesis in male F344 rat kidney. Environ Health Perspect 101(Supp1 5):249–252.

Lundberg I, Ekdahl M, Kronevi T, et al. 1986. Relative hepatotoxicity of some industrial solvents after intraperitoneal injection or inhalation exposure in rats. Environ Res 40:411–420.

Lunt RL. 1953. Delayed chloroform poisoning in obstetric practice. Br Med J 1:489–490.

McGeehin MA, Reif JS, Becher JC, et al. 1993. Case control study of bladder cancer and water disinfection methods in Colorado. Am J Epidemiol 138(7):493–501.

McKone TE. 1993. Linking a PBPK model for chloroform with measured breath concentrations in showers: implications for dermal exposure models. J Expo Anal Environ Epidemiol 3(3):339–365.

Mink FL, Brown TJ, Rickabaugh J. 1986. Absorption, distribution and excretion of C-trihalomethanes in mice and rats. Bull Environ Contam Toxicol 37:752–758.

Moore DH, Chasseaud LF, Majeed SK, et al. 1982. The effect of dose and vehicle on early tissue damage and regenerative activity after chloroform administration to mice. Food Chem Toxicol 20:95l–954.

Munson AE, Sain LE, Sanders VM, et al. 1982. Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. Environ Health Perspect 46:117–126.

Murray FJ, Schwetz BA, McBride JB, et al. 1979. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol Appl Pharmacol 50:515–522.

Nakajima T, Elovaara E, Okino T, et al. 1995. Different contributions of cytochrome P450 2E1 and P450 2B1/2 to chloroform hepatotoxicity in rat. Toxicol Appl Pharmacol 133:215–222.

Nashelsky M, Dix JD, Adelstein H, et al. 1995. Homicide facilitated by inhalation of chloroform. J Forensic Sci 40(1):134–138.

NCI. 1976. Report on carcinogenesis bioassay of chloroform. Bethesda, MD: Carcinogenesis Program, National Cancer Institute.

Newell GW, Dilley JV. 1978. Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Research Triangle Park, NC: U.S. Environmental Protection Agency, Health Effects Research Laboratory, Office of Research and Development.

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. October 14, 2005.

Palmer AK, Street AE, Roe JC, et al. 1979. Safety evaluation of toothpaste containing chloroform. II. Long term studies in rats. J Environ Pathol Toxicol 2:821–833.

Pereira MA. 1994. Route of administration determines whether chloroform enhances or inhibits cell proliferation in the liver of B6C3Fl mice. Fundam Appl Toxicol 23(1):87–92.

Pfaffenberger CD, Peoples AJ, Enos HF. 1980. Distribution of volatile halogenated organic compounds between rat blood serum and adipose tissue. Int J Environ Anal Chem 8:55–65.

Phoon WH, Goh KT, Lee LT, et al. 1983. Toxic jaundice from occupational exposure to chloroform. Med J Malaysia 38:31–34.

Piersol GM, Tumen HJ, Kau LS. 1933. Fatal poisoning following the ingestion of chloroform. Med Clin North Am 17:587–601.

Pohl LR, Branchflower RV, Highet RJ, et al. 1981. The formation of diglutathionyl dithiocarbonate as a metabolite of chloroform, bromotrichloromethane, and carbon tetrachloride. Drug Metab Dispos 9:334–339.

Reitz RH, Mendrala AL, Corley RA, et al. 1990. Estimating the risk of liver cancer associated with human exposures to chloroform using physiologically based pharmacokinetic modeling. Toxicol Appl Pharmacol 105:443–459.

Roe FJC, Palmer AK, Worden AN. 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. J Environ Pathol Toxicol 2:799–819.

Royston GD. 1924. Delayed chloroform poisoning following delivery. Am J Obstet Gynecol 10:808–814.

Ruddick JA, Villeneuve DC, Chu I. 1983. A teratological assessment of four trihalomethanes in the rat. J Environ Sci Health B18:333–349.

Schroeder HG. 1965. Acute and delayed chloroform poisoning. Br J Anaesth 37:972–975.

Schwetz BA, Leong BKJ, Gehring PJ. 1974. Embryo- and fetotoxicity of inhaled chloroform in rats. Toxicol Appl Pharmacol 28:442–451.

Smith AA, Volpitto PP, Gramling ZW, et al. 1973. Chloroform, halothane, and regional anesthesia: a comparative study. Anesth Analg 52:1–11.

Smith JH, Maita K, Sleight SD, et al. 1984. Effect of sex hormone status on chloroform nephrotoxicity and renal mixed function oxidases in mice. Toxicology 30:305–316.

Stevens JL, Anders MW. 1981. Effect of cysteine, diethyl maleate, and phenobarbital treatments on the hepatotoxicity of ^{1}H- and ^{2}H-chloroform. Chem Biol Interact 37:207–217.

Stoner GD, Conran PB, Greisiger EA, et al. 1986. Comparison of two routes of chemical administration on the lung adenoma response in strain A/J mice. Toxicol Appl Pharmacol 82:19–31.

Storms WW. 1973. Chloroform parties. J Am Med Assoc 225:160.

Taylor DC, Brown DM, Keeble R, et al. 1974. Metabolism of chloroform. II. A sex difference in the metabolism of ^{14}C-chloroform in mice. Xenobiotica 4:165–174.

Thompson DJ, Warner SD, Robinson VB. 1974. Teratology studies on orally administered chloroform in the rat and rabbit. Toxicol Appl Pharmacol 29:348–357.

Torkelson TR, Oyen F, Rowe VK. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Am Ind Hyg Assoc J 37:697–705.

Townsend E. 1939. Acute yellow atrophy of the liver. Two cases, with one recovery. Br Med J 2:558–560.

Tumasonis CF, McMartin DN, Bush B. 1985. Lifetime toxicity of chloroform and bromodichloro-methane when administered over a lifetime in rats. Ecotoxicol Environ Safety 9:233–240.

Tumasonis CF, McMartin DN, Bush B. 1987. Toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. J Environ Pathol Toxicol Oncol 7:55–64.

Wallace CJ. 1950. Hepatitis and nephrosis due to cough syrup containing chloroform. Calif Med 73:442–443.

Wang P-Y, Kaneko T, Sato A, et al. 1995. Dose and route dependent alteration of metabolism and toxicity of chloroform in fed and fasting rats. Toxicol Appl Pharm 135(1):119–126.

Wang P-Y, Kaneko T, Tsukada H, et al. 1994. Dose and route dependency of metabolism and toxicity of chloroform in ethanol-treated rats. Arch Toxicol 69:18–23.

Whitaker AM, Jones CS. 1965. Report of 1500 chloroform anesthetics administered with a precision vaporizer. Anesth Analg 44:60–65.

Withey JR, Collins BT, Collins PG. 1983. Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. J Appl Toxicol 3:249–253.

Young TB, Kanarek MS, Tsiatis AA. 1981. Epidemiologic study of drinking water chlorination and Wisconsin female cancer mortality. J Natl Cancer Inst 67:1191–1198.

Zierler S, Feingold L, Danley RA, et al. 1988. Bladder cancer in Massachusetts related to chlorinated chloraminated drinking water: a case control study. Arch Environ Health 43(2):195–200.

APPENDIX B: BACKGROUND INFORMATION FOR 1,1-DICHLOROETHYLENE

This appendix was written based primarily on the Toxicological Profile for 1,1-Dichloroethylene (ATSDR 1994). 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-dichloroethylene in humans following inhalation or oral exposure were located. Animal studies have demonstrated that 1,1-dichloroethylene 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-dichloroethylene have similarly demonstrated a rapid and near-complete absorption (Reichert et al. 1979). Doses of 1,1-dichloroethylene 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-dichloroethylene labeled with radioactive carbon (^{14}C), 81–99.8% of the administered radioactivity was recovered within 72 hours (Reichert et al. 1979), indicating a very rapid and near-complete absorption.

No studies evaluating the distribution of 1,1-dichloroethylene 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-dichloroethylene, 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-dichloroethylene for 2 hours (Jaeger et al. 1977a); fasted animals showed a higher accumulation of radiolabel than unfasted animals. 1,1-Dichloroethylene 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-dichloroethylene 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 cytochrome CYP2E1 to a reactive epoxide, 1,1-dichloroethylene oxide (Jones and Hathway 1978b; McKenna et al. 1977; Reichert et al. 1979). These metabolites 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-dichloroethylene 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-dichloroethylene 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% or less) 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-dichloroethylene 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

Following both inhalation and oral exposure, the most sensitive effects of 1,1-dichloroethylene appear to be on the liver. A preliminary study of workers exposed to 1,1-dichloroethylene for 6 years or less in a 1,1-dichloroethylene polymerization plant revealed a high incidence of hepatotoxicity; however, a full study of these workers has not been reported (EPA 1976). Numerous studies in animals have identified hepatic effects, including both biochemical changes (e.g., alterations in serum enzyme levels indicative of liver injury and induction of hepatic enzymes) and marked histological changes (e.g., midzonal and centrilobular swelling of liver, degeneration, and necrosis of hepatocytes). These effects have been

reported at acute exposure concentrations as low as 15 ppm for 23 hours/day for 5 days (Short et al. 1977c), or at higher concentrations for shorter durations (Henck et al. 1979; Jackson and Conolly 1985; Jaeger et al. 1977a, 1977b; Reitz et al. 1980; Reynolds et al. 1980; Watanabe et al. 1980). The hepatotoxic effects of 1,1-dichloroethylene following intermediate or chronic inhalation exposure in animals are similar to those described above for acute exposure (Gage 1970; Lee et al. 1977; Plummer et al. 1990; Quast et al. 1986). Using a NOAEL of 5 ppm and a LOAEL of 15 ppm for mottled livers (with increased SGPT and alkaline phosphatase activity and decreased lipid content occurring at 48 ppm) in guinea pigs exposed to 1,1-dichloroethylene for 24 hours per day for 90 days (Prendergast et al. 1967), ATSDR (1994) derived an intermediate-duration MRL of 0.02 ppm. Two chronic inhalation studies of 1,1-dichloroethylene 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-dichloroethylene is given orally, with acute effects at doses from 25 to 100 mg/kg including changes in liver serum enzymes, bile canalicular injury, and histological changes in liver cells (Andersen and Jenkins 1977; Jenkins and Andersen 1978; Kanz and Reynolds 1986; Kanz et al. 1991; Moslen et al. 1989). Chronic 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 oral MRL of 0.009 mg/kg/day for 1,1-dichloroethylene is based on a LOAEL of 9 mg/kg/day for hepatocellular changes in rats exposed *in utero* and throughout adulthood (Quast et al. 1983).

Adverse effects have been observed in the kidneys of laboratory animals following acute, intermediate, and chronic inhalation exposure to 1,1-dichloroethylene. These effects are manifested as enzyme changes (decreases in kidney monooxygenase and epoxide hydrolase levels) (Oesch et al. 1983), tubular alterations (hemoglobinuria) (McKenna et al. 1978b), gross changes (increase in organ weight) (Henck et al. 1979; Quast et al. 1986), and histological changes (tubular swelling, degeneration, and necrosis) (Henck et al. 1979; Jackson and Conolly 1985; Lee et al. 1977; McKenna et al. 1978b; Prendergast et al. 1967; Reitz et al. 1980; Short et al. 1977c; Watanabe et al. 1980). Effects have been reported in animals exposed by inhalation acutely to 10–300 ppm or chronically to 25–75 ppm (Henck et al. 1979; Maltoni et al. 1985; Prendergast et al. 1967; Quast et al. 1986; Reitz et al. 1980; Short et al. 1977b; Watanabe et al. 1980). Similar renal effects have been reported following acute 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 oral exposure to 25 mg/kg/day, an exposure level that did not produce any adverse effects (Quast et al. 1983) or chronic oral exposure to 30 mg/kg/day, an exposure level that resulted in mild hepatic effects (Rampy et al. 1977)

Following inhalation exposure in mice, rats, and rabbits, 1,1-dichloroethylene has been shown to produce effects on the developing organism, but generally only at exposure levels (15–160 ppm) that also produced maternal effects (Murray et al. 1979; Short et al. 1977a); 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-dichloroethylene via contaminated water has been published (NJDH 1992a, 1992b), but it provided only suggestive evidence of an association of 1,1-dichloroethylene with developmental effects. A single study reported no developmental effects from oral exposure of 40 mg/kg/day of 1,1-dichloroethylene 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 three-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.

Chronic occupational exposure to 1,1-dichloroethylene 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-dichloroethylene production and polymerization plant workers (Ott et al. 1976). The carcinogenicity of 1,1-dichloroethylene in laboratory animals following inhalation exposure has been evaluated in intermediate and chronic studies with rats, mice, and Chinese hamsters (Hong et al. 1981; Lee et al. 1977, 1978; Maltoni et al. 1982; Quast et al. 1986; Rampy et al. 1977; Viola and Caputo 1977). Exposure concentrations of 1,1-dichloroethylene in these studies ranged from 10 to 200 ppm. Of the long-term inhalation bioassays conducted in laboratory animals to date, only the results of a study by Maltoni et al. (1985) in mice have provided some suggestive evidence of a carcinogenic effect associated with 1,1-dichloroethylene exposure.

No studies were located regarding cancer in humans after oral exposure to 1,1-dichloroethylene. A number of chronic studies in rats and mice have evaluated the carcinogenicity of 1,1-dichloroethylene by oral exposure (Maltoni et al. 1982, 1985; NTP 1982; Ponomarkov and Tomatis 1980; Quast et al. 1983; Rampy et al. 1977) at dose levels from 0.5 to 150 mg/kg/day; a trend toward increased incidence of malignant and nonmalignant tumors in 1,1-dichloroethylene-treated animals has been reported (NTP 1982; Ponomarkov and Tomatis 1980; Quast et al. 1983), but in the majority of cases, the increase in tumor frequencies have 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-dichloroethylene is the result of biotransformation reactions and not to the parent compound (Andersen et al. 1978, 1980; Jaeger et al. 1977a; Jones and Hathway 1978c). 1,1-Dichloroethylene 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 glutathione S-transferases 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 (1994) did not derive an acute-duration inhalation MRL for 1,1-dichloroethylene.

ATSDR (1994) derived an intermediate-duration inhalation MRL of 0.02 ppm for 1,1-dichloroethylene based on a NOAEL of 5 ppm for hepatic effects in guinea pigs continuously exposed (24 hours/day, 7 days/week) to 1,1-dichloroethylene (Prendergast et al. 1967). The LOAEL was 15 ppm for mottled livers (with increased SGPT and alkaline phosphatase activity and decreased lipid content occurring at 45 ppm). The NOAEL was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) and a modifying factor of 3 was used to account for the close proximity of serious effects observed at the range of 10–25 ppm.

ATSDR (1994) did not derive a chronic-duration inhalation MRL for 1,1-dichloroethylene, citing inadequate chronic data. The chronic data of Quast et al. (1986) was not used because a serious LOAEL

of 15 ppm for developmental effects in rats and mice following acute exposure to 1,1-dichloroethylene was reported by Short et al. (1977a), which precluded derivation of a chronic-duration inhalation MRL.

ATSDR (1994) did not derive an acute-duration oral MRL for 1,1-dichloroethylene because the available suitable NOAEL of 40 mg/kg/day from a developmental toxicity study in rats (Murray et al. 1979) was too close to the 50 mg/kg single dose that was lethal in fasted rats (Andersen and Jenkins 1977).

ATSDR (1994) did not derive an intermediate-duration oral MRL for 1,1-dichloroethylene because only one study was available, in which the highest dose tested, 25 mg/kg/day, was a NOAEL (Quast et al. 1983).

ATSDR (1994) derived a chronic-duration oral MRL of 0.009 mg/kg/day based on a LOAEL of 9 mg/kg/day in rats for hepatocellular changes in a two-year exposure study (Quast et al. 1983), and using an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for animal to human extrapolations, and 10 for intrahuman variability).

EPA (IRIS 2005) derived a chronic RfD of 0.05 mg/kg/day for 1,1-dichloroethylene based on benchmark dose analysis of hepatic effects (fatty liver) in a chronic study in rats (Quast et al. 1986) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability).

EPA (IRIS 2005) derived a chronic RfC of 0.2 mg/m^3 for 1,1-dichloroethylene based on benchmark concentration analysis of hepatic effects (fatty liver) in a chronic 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 intrahuman variability).

EPA classified 1,1-dichloroethylene in Group C, *possible human carcinogen*, under the 1986 cancer guidelines (EPA 1986). Under the draft revised guidelines for carcinogen risk assessment (EPA 1996), EPA concluded that 1,1-dichloroethylene exhibits suggestive evidence of carcinogenicity but not sufficient evidence to assess human carcinogenic potential following inhalation exposure in studies in rodents. EPA (IRIS 2005) has not performed quantitative assessments of carcinogenic potential for 1,1-dichloroethylene for either the oral or inhalation route.

NTP's Eleventh Report on Carcinogens (NTP 2005) does not list 1,1-dichloroethylene. The International Agency for Research on Cancer (IARC) (1999) notes that 1,1-dichloroethylene is *not classifiable as to its carcinogenicity to humans* (Group 3).

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

The endpoints of concern for 1,1-dichloroethylene in this mixture are hepatic, renal, and developmental effects. For endpoints that are not the basis of the MRL, TTDS are derived below, using the methods described by ATSDR (2004). The derivations are based primarily on data provided in ATSDR (1994), and in particular, the LSE tables.

Inhalation TTDs

Following EPA (1994) methodology, the human equivalent concentration ($NOAEL_{HEC}$) for an extrarespiratory effect produced by a category 3 gas, such as 1,1-dichloroethylene, is calculated by multiplying the duration-adjusted animal NOAEL 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 in humans was not available (IRIS 2005), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation: The intermediate inhalation MRL for 1,1-dichloroethylene is 0.02 ppm is based on hepatic effects.

Renal Effects, Intermediate Inhalation: Maltoni et al. (1985) identified a NOAEL of 10 ppm for renal effects in mice exposed 4 hours/day, 5 days/week for 52 weeks. This duration of this study is applicable to intermediate and chronic exposure. The NOAEL was duration-adjusted to 1.2 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 1.2 ppm using the method described previously under the heading Inhalation TTDs. Application of an uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for intrahuman variability) yields a TTD_{RENAL} of 0.04 ppm.

Developmental Effects, Intermediate Inhalation: Short et al. (1977a) reported incomplete ossification in the offspring of mice exposed to 15 ppm of 1,1-dichloroethylene for 23 hours/day throughout gestation. The LOAEL of 15 ppm was duration-adjusted to 14.4 ppm for a continuous exposure scenario, and converted to a $LOAEL_{HEC}$ of 14.4 ppm 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 intrahuman variability) yields a TTD_{DEVEL} of 0.05 ppm.

Hepatic Effects, Chronic Inhalation: A $TTD_{HEPATIC}$ of 0.007 ppm is derived from the intermediate MRL based on hepatic effects; see explanation in Chapter 3.

Renal Effects, Chronic Inhalation: Maltoni et al. (1985) identified a NOAEL of 10 ppm for renal effects in mice exposed 4 hours/day, 5 days/week for 52 weeks. This duration of this study is applicable to intermediate and chronic exposure. The NOAEL was duration-adjusted to 1.2 ppm for a continuous exposure scenario, and converted to a $NOAEL_{HEC}$ of 1.2 ppm as described previously under the heading Inhalation TTDs. Application of an uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for intrahuman variability) yields a TTD_{RENAL} of 0.04 ppm.

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

Oral TTDs

Hepatic Effects, Intermediate Oral: EPA (IRIS 2005) reported NOAELs for hepatic effects of 40 mg/kg/day, 5 days/week (adjusted to 28.6 for continuous exposure) in the NTP (1982) 13-week study in rats and mice. The LOAELs for both species were 100 mg/kg/day. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability), to the NOAEL, a $TTD_{HEPATIC}$ of 0.3 mg/kg/day is estimated.

Renal Effects, Intermediate Oral: 1,1-Dichloroethylene has not been adequately tested for non-hepatic effects in intermediate-duration oral studies, but chronic 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 oral studies, and in acute and intermediate-to-chronic inhalation studies. Thus, the weight of evidence for renal effects suggests that 1,1-dichloroethylene would cause renal damage at higher doses than tested in intermediate and chronic oral studies. The intermediate oral $TTD_{HEPATIC}$ of 3.0 mg/kg/day can be adopted as an interim value for the TTD_{RENAL} for intermediate exposure.

Developmental Effects, Intermediate Oral: No developmental effects were reported in the only oral developmental toxicity study of 1,1-dichloroethylene available, which tested a single exposure level, 40 mg/kg/day, in rats on days 6–15 of gestation (Murray et al. 1979). This test was inadequate because the dose produced no effects in the dams (on body weight gain, liver weight, food, or water consumption). A three-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-dichloroethylene was developmentally toxic at exposures that also were maternotoxic, and that the mouse was the most sensitive species. Given the possibility that 1,1-dichloroethylene may also be developmentally toxic by the oral route, the intermediate oral $TTD_{HEPATIC}$ of 0.3 mg/kg/day can be adopted as an interim value for the TTD_{DEVEL} for chronic exposure.

Hepatic Effects, Chronic Oral: The chronic oral MRL of 0.009 mg/kg/day is based on hepatic effects.

Renal Effects, Chronic Oral: Chronic oral studies in animals did not report renal effects at dose levels of 1,1-dichloroethylene 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 oral studies, and in acute and intermediate-to-chronic inhalation studies. Thus, the weight of evidence suggests that 1,1-dichloroethylene may cause renal damage at higher doses than tested in intermediate and chronic oral studies. The chronic oral MRL of 0.009 mg/kg/day can be adopted as an interim value for the TTD_{RENAL} for chronic exposure.

Developmental Effects, Chronic Oral: No developmental effects were reported in the only oral developmental toxicity study of 1,1-dichloroethylene available, which tested a single exposure level, 40 mg/kg/day, in rats on days 6–15 of gestation (Murray et al. 1979). This test was inadequate because the dose produced no effects in the dams (on body weight gain, liver weight, food, or water consumption). A three-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-dichloroethylene was developmentally toxic at exposures that also were maternotoxic, and that the mouse was the most sensitive species. Given the possibility that 1,1-dichloroethylene may also be developmentally toxic by the oral route, the chronic oral MRL of 0.009 mg/kg/day can be adopted as an interim value for the TTD_{DEVEL} for chronic exposure.

Summary (TTDs for 1,1-Dichloroethylene)

Intermediate Inhalation TTDs:

$MRL_{HEPATIC}$ = 0.02 ppm
TTD_{RENAL} = 0.04 ppm
TTD_{DEVEL} = 0.05 ppm

Chronic Inhalation TTDs:

$MRL_{HEPATIC}$ = 0.007 ppm
TTD_{RENAL} = 0.04 ppm
TTD_{DEVEL} = 0.02 ppm

Intermediate Oral TTDs:

$TTD_{HEPATIC}$ = 0.3 mg/kg/day
TTD_{RENAL} = 0.3 mg/kg/day
TTD_{DEVEL} = 0.3 mg/kg/day

Chronic Oral TTDs:

$MRL_{HEPATIC}$ = 0.009 mg/kg/day
TTD_{RENAL} = 0.009 mg/kg/day
TTD_{DEVEL} = 0.009 mg/kg/day

B.6 References

Andersen ME, Jenkins J. 1977. Oral toxicity of 1,1-dichloroethylene in the rat: effects of sex, age, and fasting. Environ Health Perspect 21:157–163.

Andersen ME, Jones RA, Jenkins LJ. 1978. The acute toxicity of single, oral doses of 1,1-dichloroethylene in the fasted male rat: effect of induction and inhibition of microsomal enzyme activities on mortality. Toxicol Appl Pharmacol 46:227–234.

Andersen ME, Thomas OE, Gargas ML, et al. 1980. The significance of multiple detoxification pathways for reactive metabolites in the toxicity of 1,1-dichloroethylene. Toxicol Appl Pharmacol 52:422–432.

ATSDR. 1994. Toxicological profile for 1,1-dichloroethylene. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. February 15, 2004.

ATSDR 2004. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Chieco P, Moslen MT, Reynolds ES. 1981. Effect of administrative vehicle on oral 1,1-dichloroethylene toxicity. Toxicol Appl Pharmacol 57:146–155.

Dallas CE, Weir FW, Feldman S, et al. 1983. The uptake and disposition of 1,1-dichloroethylene in rats during inhalation exposure. Toxicol Appl Pharmacol 68:140–151.

Dekant W, Vamvakas S, Anders MW. 1989. Bioactivation of nephrotoxic haloalkenes by glutathione conjugation: formation of toxic and mutagenic intermediates by cysteine B-lyase. Drug Metab Rev 20:43–83.

D'Souza RW, Andersen ME. 1988. Physiologically based pharmacokinetic model for vinylidene chloride. Toxicol Appl Pharmacol 95:230–240.

EPA. 1976. Summary characteristics of selected chemicals of near-term interest. Washington, DC: U.S. Environmental Protection Agency. EPA5601476004.

EPA. 1986. Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. Federal Register 51(185):33992–34003.

EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency. EPA600890066F.

EPA. 1996. Proposed guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. Federal Register 61(79):17960–18011.

Forkert PG, Stringer V, Troughton KM. 1986. Pulmonary toxicity of 1,1-dichloroethylene: correlation of early changes with covalent binding. Can J Physiol Pharmacol 64:112–121.

Gage JC. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Br J Ind Med 27:1–18

Gargas ML, Andersen ME, Clewell III HJ. 1986. A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86:341–352.

Henck JW, Quast JF, Rampy LW. 1979. A comparison of four mouse strains exposed to subchronically inhaled vinylidene chloride (VDC). Toxicology Research Laboratory, Health and Environmental Science, Dow Chemical U.S.A., Midland, MI.

Hong CB, Winston JM, Thornburg LP, et al. 1981. Follow-up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: tumor incidence and mortality subsequent to exposure. J Toxicol Environ Health 7:909–924.

IARC. 1999. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71. Re-Evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide.

IRIS. 2005. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. http://www.epa.gov/iris/. October 14, 2005.

Jackson NM, Conolly RB. 1985. Acute nephrotoxicity of 1,1-dichloroethylene in the rat after inhalation exposure. Toxicol Lett 29:191–199.

Jaeger RJ, Shoner LG, Coffman LJ. 1977a. 1,1-Dichloroethylene hepatotoxicity: proposed mechanism of action and distribution and binding of ^{14}C radioactivity following inhalation exposure in rats. Environ Health Perspect 21:113–119.

Jaeger RJ Szabo S, Coffman LJ. 1977b. 1,1-Dichloroethylene hepatotoxicity: effect of altered thyroid function and evidence for the subcellular site of injury. J Toxicol Environ Health 3:545–555.

Jenkins LJ, Andersen ME. 1978. 1,1-Dichloroethylene nephrotoxicity in the rat. Toxicol Appl Pharmacol 46:131–141.

Jones BK, Hathway DE. 1978a. Differences in metabolism of vinylidene chloride between mice and rats. Br J Cancer 37:411–417.

Jones BK, Hathway DE. 1978b. The biological fate of vinylidene chloride in rats. Chem Biol Interact 20:27–41.

Jones BK, Hathway DE. 1978c. Tissue-mediated mutagenicity of vinylidene chloride in *Salmonella typhimurium* TA1535. Cancer Lett 5:1–6.

Kanz MF, Reynolds ES. 1986. Early effects of 1,1-dichloroethylene on canalicular and plasma membranes: ultrastructure and stereology. Exp Mel Pathol 44:93–110.

Kanz MF, Taj Z, Moslen MT. 1991. 1,1-Dichloroethylene hepatotoxicity: hypothyroidism decreases metabolism and covalent binding but not injury in the rat. Toxicology 70:213–229.

Lee CC, Bhandari JC, Winston JM, et al. 1977. Inhalation toxicity of vinyl chloride and vinylidene chloride. Environ Health Perspect 21:25–32.

Lee CC, Bhandari JC, Winston JM, et al. 1978. Carcinogenicity of vinyl chloride and vinylidene chloride. J Toxicol Environ Health 4:15–30.

Maltoni C, Ciliberti A, Carretti D. 1982. Experimental contributions in identifying brain potential carcinogens in the petrochemical industry. Ann NY Acad Sci 3811:216–249.

Maltoni C, Lefemine P, Cotti G, et al., eds. 1985. Experimental research on vinylidene chloride carcinogenesis: archives of research on industrial carcinogenesis. Volume 3. Princeton, NJ: Princeton Scientific Publishers.

McKenna MJ, Watanabe PG, Gehring PG. 1977. Pharmacokinetics of vinylidene chloride in the rat. Environ Health Perspect 21:99–105.

McKenna MJ, Zempel JA, Madrid EO, et al. 1978a. Metabolism and pharmacokinetic profile of vinylidene chloride in rats following oral administration. Toxicol Appl Pharmacol 45:821–835.

McKenna MJ, Zempel JA, Madrid EO, et al. 1978b. The pharmacokinetics of ^{14}C vinylidene chloride in rats following inhalation exposure. Toxicol Appl Pharmacol 45:599–610.

Moslen MT, Whitehead RF, Ferguson AE, et al. 1989. Protection by L-2-oxothiazolidine-4-carboxylate, a cysteine prodrug, against 1,1-dichloroethylene hepatotoxicity in rats is associated with decreases in toxin metabolism and cytochrome P-450. J Pharmacol Exp Ther 248(1):157–163.

Murray FJ, Nitschke KD, Rampy LW, et al. 1979. Embryotoxicity and fetotoxicity of inhaled or ingested vinylidene chloride in rats and rabbits. Toxicol Appl Pharmacol 49:189–202.

Nitschke KD, Smith FA, Quast JM, et al. 1983. A three-generation rat reproductive toxicity study of vinylidene chloride in the drinking water. Fundam Appl Toxicol 3:75–79.

NJDH. 1992a. Population-based surveillance and etiological research of adverse reproductive outcomes and toxic wastes; Report on Phase IV-A: Public drinking water contamination and birthweight, fetal deaths, and birth defects; A cross-sectional study. Trenton, NJ: New Jersey Department of Health, jointly published with the Agency for Toxic Substances and Disease Registry. April 1992.

NJDH. 1992b. Population-based surveillance and etiological research of adverse reproductive outcomes and toxic wastes; Report on Phase IV-B: Public drinking water contamination and birthweight, fetal deaths, and birth defects; A cross-sectional study. Trenton, NJ: New Jersey Department of Health, jointly published with the Agency for Toxic Substances and Disease Registry. April 1992.

NTP. 1982. National Toxicology Program--Technical report series No. 228. Carcinogenesis bioassay of vinylidene chloride (CAS No. 75-35-4) in F344 rats and B6C3Fl mice (gavage study). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Services, National Institutes of Health. NIH Publication No. 82-1784.

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. October 14, 2005.

Oesch F, Protic-Sabljic M, Friedberg T, et al. 1983. Vinylidene chloride: changes in drug metabolizing enzymes, mutagenicity and relation to its targets for carcinogenesis. Carcinogenesis 4:1031–1038.

Ott MG, Fishbeck WA, Townsend JC, et al. 1976. A health study of employees exposed to vinylidene chloride. J Occup Med 18:735–738.

Plummer JL, Hall PM, Ilsley AH, et al. 1990. Influence of enzyme induction and exposure profile on liver injury due to chlorinated hydrocarbon inhalation. Pharmacol Toxicol 67:329–335.

Ponomarkov V, Tomatis L. 1980. Long-term testing of vinylidene chloride and chloroprene for carcinogenicity in rats. Oncology 37:136–141.

Prendergast JA, Jones RA, Jenkins LJ, et al. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol Appl Pharmacol 10:270–289.

Putcha L, Bruchner JV, D'Soyza R, et al. 1986. Toxicokinetics and bioavailability of oral and intravenous 1,1-dichloroethylene. Fundam Appl Toxicol 6:240–250.

Quast JF, Humiston CG, Wade CE, et al. 1983. A chronic toxicity and oncogenicity study in rats and subchronic toxicity study in dogs on ingested vinylidene chloride. Fundam Appl Toxicol 3(1):55–62.

Quast JF, McKenna MJ, Rampy LW, et al. 1986. Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats. Fundam Appl Toxicol 6:105–144.

Rampy LW, Quast JF, Humiston CG, et al. 1977. Interim results of two-year toxicological studies in rats of vinylidene chloride incorporated in the drinking water or administered by repeated inhalation. Environ Health Perspect 21:33–43.

Ramsey JC, Andersen ME. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73:159–175.

Reichert D, Werner HE, Metzler M, et al. 1979. Molecular mechanism of 1,1-dichloroethylene toxicity: excreted metabolites reveal different pathways of reactive intermediates. Arch Toxicol 42:159–169.

Reitz RH, Watanabe PG, McKenna MJ, et al. 1980. Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: a comparative study with dimethylnitrosamine. Toxicol Appl Pharmacol 52:357–370.

Reynolds ES, Moslen MT, Boor PJ, et al. 1980. 1,1-Dichloroethylene hepatotoxicity. Am J Pathol 101(2):331–344.

Short RD, Minor JL, Winston JM, et al. 1977a. Toxicity studies of selected chemicals: Task II. The development of vinylidene chloride inhaled by rats and mice during gestation. Washington, DC: U.S. Environmental Protection Agency. EPA-560/6-77-022.

Short RD Winston JM, Minor JL, et al. 1977b. Effect of various treatments on toxicity of inhaled vinylidene chloride. Environ Health Perspect 21:125–129.

Short RD, Winston JM, Minor JL, et al. 1977c. Toxicity of vinylidene chloride in mice and rats and its alteration by various treatments. J Toxicol Environ Health 3:913–921.

Vamvakas S, Anders MW. 1990. Formation of reactive intermediates by phase II enzymes: glutathione-dependent bioactivation reactions. In: Witmer CM, ed. Advances in experimental medicine and biology. New York, NY: Plenum Publishing Co., 13–24.

Viola PL, Caputo A. 1977. Carcinogenicity studies on vinylidene chloride. Environ Health Perspect 21:45–47.

Watanabe PG, Reitz RH, Schumann AM, et al. 1980. Implications of the mechanisms of tumorgenicity for risk assessment. In: Witschi M, ed. The scientific basis of toxicity assessment. Amsterdam: Elsevier, Volume 6, 69–81.

Waxweiler R. 1981. Epidemiologic problems associated with exposure to several agents. Environ Health Perspect 42:51–56.

APPENDIX C: BACKGROUND INFORMATION FOR TRICHLOROETHYLENE

This appendix was written based primarily on the Toxicological Profile for Trichloroethylene (ATSDR 1997). 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.

C.1 Toxicokinetics

Studies of humans and rats indicate that inhaled trichloroethylene is rapidly and efficiently absorbed (ATSDR 1997). 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 1997). 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 1997). 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 1997). 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 predominately 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 1997). 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 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 1997; Lash et al. 2000). Trichloro-

ethylene 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 CYP 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 CYP 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 γ-glutamyl transferase 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.

Minor metabolic pathways arising from the initial intermediate, trichloroethylene oxide, or the trichloroethylene-CYP 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 1997; 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 DNA leading to kidney cytotoxicity and kidney tumor development.

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 1997; Clewell et al. 2000; Fisher 2000). 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

Results from studies of trichloroethylene-exposed humans and animals indicate that the primary targets for trichloroethylene's noncarcinogenic toxicity are the nervous system, liver, heart, and kidneys (ATSDR 1997). The critical target (i.e., the target in which effects occur at the lowest exposure level) is expected to be the nervous system. Studies involving acute- or intermediate-duration inhalation or oral exposures have observed changes in neurobehavior in humans and animals at lower exposure levels (50–200 ppm) than those associated with liver effects (liver enlargement and cellular hypertrophy) and kidney effects (increased kidney weights and cytomegaly and karyomegaly in renal tubular epithelial cells) observed in animal studies (ATSDR 1997). For example, Stewart et al. (1970) found no changes in liver function tests in humans who were exposed to 200 ppm for 7 hours/day for 5 days and reported experiencing headache, fatigue, and drowsiness. Effects on the heart appear to be restricted to cardiac arrhythmias due to trichloroethylene sensitization of the heart to epinephrine and other catecholamines, and appear to be a high exposure/dose phenomenon. Additional endpoints of concern are immunological effects and effects on the developing organism. There is suggestive but inconclusive evidence in humans for these effects (ATSDR 1997). In animal studies, evidence of immunotoxicity (Aranyi et al. 1986; Sanders et al. 1982) and evidence of developmental toxicity (ATSDR 1997; Dorfmueller et al. 1979; Isaacson et al. 1989) has also been reported from both these routes of exposure.

Occupational exposure to trichloroethylene has been widespread due to its use in dry cleaning, for metal degreasing, and as a solvent for oils and resins. A recent article (Wartenberg et al. 2000) reviewed over 80 published papers and letters on the epidemiology of cancer in groups of people occupationally exposed to trichloroethylene. Elevated relative risks, ranging from 1.1 to 2.0, have been reported for kidney cancer, liver cancer, and non-Hodgkin's lymphoma in several cohorts of workers repeatedly exposed to trichloroethylene in workplace air (see Wartenberg et al. 2000). Workers in these studies, however, were also exposed to other solvents (e.g., tetrachloroethylene). Accurate adjustment for this and other confounding factors is not possible from the available data. Wartenberg et al. (2000) concluded that there is "moderate support" for a causative relationship between exposure to trichloroethylene and cancer using Hill's criteria of causation. Reflecting this assessment, IARC (1995) earlier concluded that the human evidence for trichloroethylene carcinogenicity is limited.

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 1997).

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 do rats. In contrast, rats appear to be uniquely susceptible to trichloroethylene-induced kidney damage and tumors. Based on its review of available data, ATSDR (1997) 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. EPA-supported monographs on trichloroethylene health risks have been recently published and are being used to develop updated EPA health risk characterizations for trichloroethylene (Scott and Cogliano 2000).

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, Mikiskova and Mikiska (1966) reported that intraperitoneally administered trichloroethanol was 5–6 times more effective than trichloroethylene in altering electrophysiological variables associated with central nervous system depression in guinea pigs. Blain et al. (1992) found that effects on electrophysiological endpoints in rabbits exposed to trichloroethylene by inhalation correlated better with blood levels of trichloroethanol than trichloroethylene.

Metabolism of trichloroethylene is expected to produce cytotoxic and carcinogenic metabolites, including trichloroacetic acid, dichloroacetic acid, chloral hydrate, and 2-chloroacetaldehyde (ATSDR 1997). 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 CYP 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 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 1997).

Trichloroethylene-induced cardiac arrhythmias are thought to involve parent-chemical sensitization of the heart to epinephrine-induced arrhythmias (ATSDR 1997). 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 (1997) derived an acute inhalation MRL of 2 ppm for trichloroethylene based on a LOAEL of 200 ppm for subjective neurological symptoms such as fatigue and drowsiness in volunteers exposed 7 hours/day for 5 days (Stewart et al. 1970) and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 to account for human variability).

ATSDR (1997) derived an intermediate-duration inhalation MRL of 0.1 ppm for trichloroethylene based on a LOAEL of 50 ppm for decreased wakefulness during exposure, decreased postexposure heart rate, and slow-wave sleep in rats exposed for 8 hours/day, 5 days/week for 6 weeks (Arito et al. 1994), and an uncertainty factor of 300 (10 for using a LOAEL, 3 for extrapolating from rats to humans, and 10 to account for human variability).

ATSDR (1997) did not derive a chronic inhalation MRL for trichloroethylene due to the lack of suitable data.

ATSDR (1997) derived an acute oral MRL of 0.2 mg/kg/day for trichloroethylene based on a LOAEL of 50 mg/kg/day for reduced rearing rate in mice and an uncertainty factor of 300 (10 for the use of a LOAEL, 10 for extrapolating from animals to humans, and 3 for human variability [a full factor of 10 was not used because pups were taken to represent a sensitive population]). The mice were exposed for

7 days beginning at 10 days of age and evaluated for locomotion, rearing, and total activity at 17 and 60 days of age; the effect was seen at 60 days of age (Fredriksson et al. 1993).

ATSDR (1997) did not derive intermediate or chronic oral MRLs for trichloroethylene due to the lack of suitable data.

NTP (2005) listed trichloroethylene as *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of malignant tumor formation in experimental animals, and convincing relevant information that trichloroethylene acts through mechanisms indicating it would likely cause cancer in humans. IARC (1995) assigned trichloroethylene to Cancer Group 2A, *probably carcinogenic to humans*, based on limited evidence in humans and sufficient evidence in experimental animals. IARC (1995) noted that (1) although a hypothesis linking the formation of mouse liver tumors with peroxisome proliferation is plausible, trichloroethylene also induced tumors at other sites in mice and rats, and (2) several epidemiological studies showed elevated risks for cancer of the liver and biliary tract and for non-Hodgkin's lymphoma. EPA's Integrated Risk Information System (IRIS) database (IRIS 2005) does not list an RfD, RfC, or a carcinogenicity assessment for trichloroethylene. As reviewed by ATSDR (1997), the EPA Scientific Advisory Board in 1988 offered the opinion that the weight of evidence for trichloroethylene carcinogenicity was on a Group B2/C continuum (i.e., on the border between Group B2 and Group C). EPA has not yet presented a more recent position on the weight-of-evidence classification for trichloroethylene carcinogenicity, but is currently evaluating several approaches to extrapolating from the animal tumor data for trichloroethylene to derive estimates of human cancer risks at environmentally relevant exposure levels (Scott and Cogliano 2000).

C.5 Derivation of Target Organ Toxicity Dose (TTD) Values

The 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 (2004). The derivations are based primarily on data provided in ATSDR (1997), and in particular, the LSE tables

Inhalation TTDs

Following EPA (1994) methodology, the human equivalent concentration ($NOAEL_{HEC}$) for an extrarespiratory effect produced by a category 3 gas, such as trichloroethylene, is calculated by multiplying the duration-adjusted animal NOAEL by the ratio of the blood:gas partition coefficients in

animals and humans [(Hb/g)A / Hb/g)H]. Since the partition coefficients 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 24 hours/day for 30 days. The NOAEL is converted to a $NOAEL_{HEC}$ 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 intrahuman variability) was applied to the $NOAEL_{HEC}$ to derive the $TTD_{HEPATIC}$ of 1 ppm.

Renal Effects, Intermediate Inhalation: Intermediate duration studies identified NOAELs but no LOAELs for renal effects in animals exposed to chloroform by inhalation. Maltoni et al. (1988) reported a NOAEL of 100 ppm and a LOAEL of 300 ppm for renal tubule meganucleocytosis 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 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 and 10 for intrahuman variability) was applied to the $NOAEL_{HEC}$ to derive the TTD_{RENAL} of 0.7 ppm.

Immunological Effects, Intermediate Inhalation: There are some indications of immune abnormalities in occupationally exposed persons (dermal sensitivity reactions) and in limited studies of populations exposed to contaminated drinking water, but the evidence is inconclusive (ATSDR 1997). Immunological effects have not been reported in intermediate or chronic duration studies of inhaled trichloroethylene in animals. Increased susceptibility to pulmonary infection with *Streptococcus zooepidemicus* occurred in mice by inhalation exposed to ≥10 ppm of trichloroethylene for 3 hours (Aranyi et al. 1986), and acute and intermediate studies of oral exposure to trichloroethylene in mice reported suppression of humoral and cellular immunity (Sanders et al. 1982). Therefore, the weight of evidence suggests that trichloroethylene may be immunotoxic, and the intermediate duration MRL of 0.1 ppm can be adopted as an interim value for the TTD_{IMMUNO}.

Neurological Effects, Intermediate Inhalation: The intermediate inhalation MRL of 0.1 ppm for trichloroethylene is based on neurological effects.

Developmental Effects, Intermediate Inhalation: While no single study has identified both a NOAEL and a LOAEL for developmental effects following inhalation of trichloroethylene, Beliles et al. (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. The NOAEL of 500 ppm was therefore selected, and duration-adjusted (from 7 hours/day, 5 days/week) to 104 ppm for continuous exposure. The NOAEL was converted to a $NOAEL_{HEC}$ of 104 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 intrahuman variability) was then applied to derive the TTD_{DEVEL} of 3 ppm.

Hepatic Effects, Chronic Inhalation: A $TTD_{HEPATIC}$ of 0.3 ppm is derived from the intermediate $TTD_{HEPATIC}$; 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 meganucleocytosis 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 intrahuman variability) was applied to the $NOAEL_{HEC}$ to derive the TTD_{RENAL} of 0.7 ppm.

Immunological Effects, Chronic Inhalation: A TTD_{IMMUNO} of 0.03 ppm is derived from the intermediate TTD_{IMMUNO}; see explanation in Chapter 3.

Neurological, Chronic Inhalation: A TTD_{NEURO} of 0.03 ppm is derived from the intermediate MRL; see explanation in Chapter 3.

Developmental Effects, Chronic Inhalation: A TTD_{DEVEL} of 1 ppm is derived from the intermediate TTD_{DEVEL}; see explanation in Chapter 3.

Oral TTDs

Hepatic Effects, Intermediate Oral: Chronic studies of trichloroethylene toxicity have failed to report hepatic effects, even at doses as high as 1,000 mg/kg/day. The highest subchronic NOAEL that is still below available subchronic LOAEL values is from the study of Stott et al. (1982) who exposed B6C3F1 mice to trichloroethylene by gavage for 5 days/week for 3 weeks. The study identified a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day for increased hepatic DNA content/gram of tissue. This NOAEL value and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) were used to derive the TTD of 3 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 renal effects (elevated urinary protein and ketone) from 6 months of exposure to trichloroethylene in the drinking water. Application of an uncertainty factor of 100 (10 for animal to human extrapolations, 10 for intrahuman variability) results in a TTD_{RENAL} of 2 mg/kg/day.

Immunological Effects, Intermediate Oral: There are some indications of immune abnormalities in limited studies of populations exposed to contaminated drinking water, but the evidence is inconclusive (ATSDR 1997). Sanders et al. (1982) reported a NOAEL of 200 mg/kg/day and a LOAEL of 400 mg/kg/day of trichloroethylene for suppressed humoral and cellular immunity in mice exposed in the drinking water for 4–6 months. To the NOAEL of 200 mg/kg/day, an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) was applied to give the TTD_{IMMUNO} of 2 mg/kg/day.

Neurological Effects, Intermediate Oral: ATSDR (1997) has developed an acute-duration (14 days or less) MRL of 0.2 mg/kg/day for trichloroethylene based on a LOAEL for behavioral changes in mice administered 50 mg/kg/day by gavage for 7 days starting at 10 days of age; effects were seen at 60 days of age. This LOAEL is similar to the LOAEL of 37 mg/kg/day in an intermediate-duration developmental study for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (ATSDR 1997). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. ATSDR (1997), however, also mentions a LOAEL of 23.3 mg/kg/day for behavioral effects and decreased brain myelination in adult rats exposed to trichloroethylene in their drinking water for 4 weeks, followed by 2 weeks of nonexposure, and 2 more weeks of exposure (Isaacson et al. 1990). Chronic oral studies in rats and mice have reported overt signs of neurotoxicity, but have only examined higher dose levels. Using the LOAEL of 23.3 mg/kg/day for adult rats and an uncertainty factor of 300 (10 for LOAEL, 10 for species extrapolation, and 3 for human variability because a potentially sensitive subpopulation has been tested) would result in a TTD_{NEURO} of 0.08 mg/kg/day.

Developmental Effects, Intermediate Oral: The lowest oral LOAEL for developmental effects reported by ATSDR (1997) is 37 mg/kg/day for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (Isaacson and Taylor 1989). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. To this LOAEL an uncertainty factor of 1,000 (10 for the

use of a LOAEL, 10 for species extrapolation, and 10 for human variability because pups represent a sensitive subpopulation) was applied, resulting in a TTD_{DEVEL} of 0.1 mg/kg/day.

Hepatic Effects, Chronic Oral: Chronic studies of trichloroethylene toxicity have failed to report hepatic effects, even at doses as high as 1,000 mg/kg/day. The highest subchronic NOAEL that is still below available subchronic LOAEL values is from the study of Stott et al. (1982) who exposed B6C3F1 mice to trichloroethylene by gavage for 5 days/week for 3 weeks. The study identified a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day for increased hepatic DNA content/gram of tissue. This NOAEL value and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) were used to derive the TTD of 3 mg/kg/day for hepatic effects.

Renal Effects, Chronic Oral: Chronic studies of trichloroethylene have reported kidney effects in rats and mice (NCI 1976; NTP 1988, 1990). The lowest LOAEL was 500 mg/kg/day, 5 days/week; a NOAEL was not defined. Tucker et al. (1982) reported a NOAEL of 217 mg/kg/day and a LOAEL of 393 mg/kg/day in male mice for renal effects (elevated urinary protein and ketone) from 6 months of exposure to trichloroethylene in the drinking water. Application of an uncertainty factor of 100 (10 for animal to human extrapolations, 10 for intrahuman variability) results in a TTD_{RENAL} of 2 mg/kg/day.

Immunological Effects, Chronic Oral: Sanders et al. (1982) reported a NOAEL of 200 mg/kg/day and a LOAEL of 400 mg/kg/day of trichloroethylene for suppressed humoral and cellular immunity in mice exposed in the drinking water for 4–6 months. To the NOAEL of 200 mg/kg/day, an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for intrahuman variability) was applied to give the TTD_{IMMUNO} of 2 mg/kg/day. The duration of exposure was judged sufficient to be applicable to chronic as well as to intermediate exposure.

Neurological, Chronic Oral: ATSDR (1997) has developed an acute-duration (14 days or less) MRL of 0.2 mg/kg/day for trichloroethylene based on a LOAEL for behavioral changes in mice administered 50 mg/kg/day by gavage for 7 days starting at 10 days of age; effects were seen at 60 days of age. This LOAEL is similar to the LOAEL of 37 mg/kg/day in an intermediate-duration developmental study for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (ATSDR 1997). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. ATSDR (1997), however, also mentions a LOAEL of 23.3 mg/kg/day for behavioral effects and decreased brain myelination in adult rats exposed to trichloroethylene in their drinking water identified by Isaacson et al. (1990). Chronic oral studies in rats and mice have reported overt signs of neurotoxicity,

but have only examined higher dose levels. Using the LOAEL of 23.3 mg/kg/day for adult rats and an uncertainty factor of 300 (10 for LOAEL, 10 for species extrapolation, and 3 for human variability because a potentially sensitive subpopulation has been tested) would result in a TTD_{NEURO} of 0.08 mg/kg/day. Because of the short duration of exposure (4 weeks, followed by 2 weeks of nonexposure, and 2 more weeks of exposure), and the lack of investigation of dose-response relationships for sensitive neurological endpoints in chronic oral studies, an additional uncertainty factor of 10 for extrapolation to chronic exposure is appropriate. The total uncertainty factor of 3,000 results in a TTD_{NEURO} of 0.008 mg/kg/day.

Developmental Effects, Chronic Oral: The lowest oral LOAEL for developmental effects reported by ATSDR (1997) is 37 mg/kg/day for behavioral changes at 60 days of age and a decreased number of myelinated fibers in the hippocampus at 21 days of age in rats exposed through their dams (Isaacson and Taylor 1989). The dams were exposed to trichloroethylene in their drinking water starting 14 days before mating and throughout gestation and lactation. To this LOAEL an uncertainty factor of 300 (10 for the use of a LOAEL, 10 for species extrapolation, and 3 for human variability because pups represent a sensitive subpopulation) was applied, resulting in a TTD_{DEVEL} of 0.1 mg/kg/day.

Summary (TTDs for Trichloroethylene)

Intermediate Inhalation TTDs:

$TTD_{HEPATIC}$ = 1 ppm
TTD_{RENAL} = 0.7 ppm
TTD_{IMMUNO} = 0.1 ppm
MRL_{NEURO} = 0.1 ppm
TTD_{DEVEL} = 3 ppm

Chronic Inhalation TTDs:

$TTD_{HEPATIC}$ = 0.3 ppm
TTD_{RENAL} = 0.7 ppm
TTD_{IMMUNO} = 0.03 ppm
MRL_{NEURO} = 0.03 ppm
TTD_{DEVEL} = 1 ppm

Intermediate Oral TTDs:

$TTD_{HEPATIC}$ = 3 mg/kg/day
TTD_{RENAL} = 2 mg/kg/day
TTD_{IMMUNO} = 2 mg/kg/day
MRL_{NEURO} = 0.08 mg/kg/day
TTD_{DEVEL} = 0.1 mg/kg/day

Chronic Oral TTDs:

$TTD_{HEPATIC}$ = 3 mg/kg/day
TTD_{RENAL} = 2 mg/kg/day
TTD_{IMMUNO} = 2 mg/kg/day
MRL_{NEURO} = 0.008 mg/kg/day
TTD_{DEVEL} = 0.1 mg/kg/day

C.6 References

Allemand H, Pessayre D, Descatoire V, et al. 1978. Metabolic activation of trichloroethylene into a chemically reactive metabolite toxic to the liver. J Pharmacol Exp Ther 204(3):714–723.

Aranyi C, O'Shea WJ, Graham JA, et al. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6:713–720.

Arito H, Takahashi M, Ishikawa T. 1994. Effect of subchronic inhalation exposure to low-level trichloroethylene on heart rate and wakefulness-sleep in freely moving rats. Sangyo Igaku 36:1–8.

ATSDR. 1997. Toxicological profile for trichloroethylene. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

ATSDR 2004. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Barton HA, Clewell HJ. 2000. Evaluating noncancer effects of trichloroethylene: dosimetry, mode of action, and risk assessment. Environ Health Perspect 108:323–334.

Beliles RP, Brucik DJ, Mecler FJ, et al. 1980. Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide. U.S. Department of Health, Education and Welfare. Contract No. 210-77-0047.

Blain L, Lachapelle P, Molotchnikoff. 1992. Evoked potentials are modified by long term exposure to trichloroethylene. Neurotoxicology 13:203–206. (As cited in ATSDR 1997 and PubMed.)

Bull RJ. 2000. Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. Environ Health Perspect 108:241–259.

Clewell HJ, Gentry PR, Covington TR, et al. 2000. Development of a physiologically based pharmacokinetic model of trichloroethylene and its metabolites for use in risk assessment. Environ Health Perspect 108:283–305.

Dorfmueller MA, Henne SP, York RG, et al. 1979. Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene. Toxicology 14:153–166.

D'Souza RW, Bruckner JV, Feldman S. 1985. Oral and intravenous trichloroethylene pharmacokinetics in the rat. J Toxicol Environ Health 15:587–601.

EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency. EPA600890066F.

Fisher JW. 2000. Physiologically based pharmacokinetic models for trichloroethylene and its oxidative metabolites. Environ Health Perspect 108(Suppl 2):265–273.

Fredriksson A, Danielsson BRG, Eriksson P. 1993. Altered behavior in adult mice orally exposed to tri- and tetrachloroethylene as neonates. Toxicol Lett 66:13–19.

Green T. 2000. Pulmonary toxicity and carcinogenicity of trichloroethylene: species differences and modes of action. Environ Health Perspect 108:261–264.

Hardin BD, Bond GP, Sikov MR, et al. 1981. Testing of selected workplace chemicals for teratogenic potential. Scand J Work Environ Health 7(Suppl 4):66–75.

IARC. 1995. Monographs on the evaluation of carcinogenic risks to humans. Volume 63. Dry-cleaning, chlorinated solvent, and other industrial chemicals. Lyon, France: World Health Organization.

IRIS. 2005. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. http://www.epa.gov/iris/. October 14, 2005.

Isaacson LG, Taylor DH. 1989. Maternal exposure to 1,l-trichloroethylene affects myelin in the hippocampal formation of the developing rat. Brain Res 488:403–407.

Isaacson LG, Spohler SA, Taylor DH. 1990. Trichloroethylene affects learning and decreases myelin in the rat hippocampus. Neurotoxicol Teratol 12:375–381.

Kjellstrand P, Holmquist B, Alm P, et al. 1983. Trichloroethylene: further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice. Acta Pharmacol Toxicol 53:375–384.

Koizumi A, Kastl PE Reitz RH, et al. 1986. Fate of ^{14}C-trichloroethylene administered to rats in drinking water. Midland, Michigan: DOW Chemical USA. Health and Environmental Sciences. Mammalian and Environmental Toxicology.

Lash LH, Fisher JW, Lipscomb JC, et al. 2000. Metabolism of trichloroethylene. Environ Health Perspect 108:177–200.

Maltoni C, Lefemine G, Cotti G, et al. 1988. Long-term carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F mice. Ann NY Acad Sci 534:316–342.

Mikiskova H, Mikiska A. 1966. Trichloroethanol in trichloroethylene poisoning. Br J Ind Med 23:116–125.

Monster AC, Boersma G, Duba WC. 1976. Pharmacokinetics of trichloroethylene in volunteers: influence of workload and exposure concentration. Int Arch Occup Environ Health 38:87–102.

Monster AC, Boersma G, Duba WC. 1979. Kinetics of trichloroethylene in repeated exposure of volunteers. Int Arch Occup Environ Health 42:283–292.

Moslen MT, Reynolds ES, Szabo S. 1977. Enhancement of the metabolism and hepatotoxicity of trichloroethylene and perchloroethylene. Biochem Pharmacol 26:369–375.

Nakajima T, Wang R-S, Murayama N, et al. 1990. Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. Toxicol Appl Pharmacol 102:546–552.

NCI. 1976. Carcinogenesis bioassay of trichloroethylene (CAS No. 79-01-6). Bethesda, MD: National Cancer Institute, Division of Cancer Cause and Prevention, Carcinogenesis Program, Carcinogen Bioassay and Program Resources Branch. NCI-CG-TR-2, DHEW Publication No. (NIH) 76-802.

NTP. 1988. National Toxicology Program--technical report series no. 273. Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH publication no. 88-2525.

NTP. 1990. National Toxicology Program--technical report series no. 243. Carcinogenesis studies of trichloroethylene (without epichlorohydrinin) (CAS No. 79-01-6) in Fischer-344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH publication no. 90-1799.

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. October 14, 2005.

Rhomberg LR. 2000. Dose–response analyses of the carcinogenic effects of trichloroethylene in experimental animals. Environ Health Perspect 108:343–358.

Sanders VM, Tucker AN, White KL Jr, et al. 1982. Humoral and cell-mediated immune status in mice exposed to trichloroethylene in drinking water. Toxicol Appl Pharmaco 62:358–368.

Savolainen H, Pfaffli P, Tengen M, et al. 1977. Trichloroethylene and 1,1,1-trichloroethane: effects on brain and liver after five days intermittent inhalation. Arch Toxicol 38:229–237.

Scott CS, Cogliano VJ. 2000. Trichloroethylene health risks—State of the science. Environ Health Perspect 108:159–160.

Stewart RD, Dodd HC, Gay HH, et al. 1970. Experimental human exposure to trichloroethylene. Arch Environ Health 20:64–71.

Stott WT, Quast JF, Watanabe PG. 1982. Pharmacokinetics and macromolecular interaction of trichloroethylene in mice and rats. Toxicol Appl Pharmacol 62:137–151.

Tucker AN, Sanders VM, Barnes DW, et al. 1982. Toxicology of trichloroethylene in the mouse. Toxicol Appl Pharmacol 62:351–357.

Wartenberg D, Reyner D, Scott CS. 2000. Trichloroethylene and cancer: epidemiologic evidence. Environ Health Perspect 108:161–176.

APPENDIX D: BACKGROUND INFORMATION FOR VINYL CHLORIDE

This appendix was written based primarily on the Toxicological Profile for Vinyl Chloride (ATSDR 2004b). 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 glutathione S-transferase enzymes. The conjugated products are excreted in urine as substituted cysteine derivatives and include thiodiglycolic acid, S-formyl-methylcysteine, and N-acetyl-S-(2-hydroxy-ethyl)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 exposure; 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 on IRIS (2005) was used to derive the chronic-duration MRL, based on exposures from the Til et al. (1983, 1991) dietary study. For additional details on PBPK models, see ATSDR (2004b).

D.2 Health 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 (Berk et al. 1975; Falk et al. 1974; Gedigke et al. 1975; Ho et al. 1991; Jones and Smith 1982; Lilis et al. 1975; Liss et al. 1985; Marsteller et al. 1975; NIOSH 1977; Popper and Thomas 1975; Suciu et al. 1975; Tamburro et al. 1984; Vihko et al. 1984). 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; Suciu 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 exposure to vinyl chloride by inhalation has also been demonstrated to result in hepatic cancer, specifically angiosarcoma (Fortwengler et al. 1999; Fox and Collier 1977; Infante et al. 1976; Jones et al. 1988; Rinsky et al. 1988; Weber et al. 1981; Wong et al. 1991; Wu et al. 1989).

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). 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 (John et al. 1977, 1981;

Mirkova et al. 1978; Sal'nikova and Kotsovskaya 1980; Ungvary et al. 1978), but only at concentrations that are also toxic to maternal animals. 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. No studies of developmental effects following oral exposure in humans or animals were located.

The most commonly reported central nervous system effects of vinyl chloride inhalation in humans are ataxia or dizziness (Ho et al. 1991; Langauer-Lewowicka et al. 1983; Lilis et al. 1975; Marsteller et al. 1975; Spirtas et al. 1975; Suciu et al. 1963, 1975; Veltman et al. 1975), drowsiness or fatigue (Langauer-Lewowicka et al. 1983; Spirtas et al. 1975; Suciu et al. 1963, 1975; Walker 1976), loss of consciousness (NIOSH 1977), and/or headache (Langauer-Lewowicka et al. 1983; Lilis et al. 1975; Marsteller et al. 1975; NIOSH 1977; Spirtas et al. 1975; Suciu et al. 1963, 1975; Veltman et al. 1975). Other central nervous system 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). Central nervous system 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 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.

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), and complement activation (Grainger et al. 1980; Ward 1976). 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 immunoglobulin G (IgG) has been obtained, and it has been proposed that vinyl chloride or a metabolite binds to IgG (Grainger et al. 1980). No studies of immunological effects of oral exposure to vinyl chloride in humans or animals were located.

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 (Byren et al. 1976; Creech and Johnson 1974; Fox and Collier 1977; Infante et al. 1976; Jones et al. 1988; Laplanche et al. 1992; Lee et al. 1996; Monson et al. 1975; Pirastu et al. 1990; Rinsky et al. 1988; Simonato et al. 1991; Teta et al. 1990; Theriault and Allard 1981; Waxweiler et al. 1976; Weber et al. 1981; Wong et al. 1991; Wu et al. 1989). 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 (Cheng et al. 1999; Du and Wang 1998; Lelbach 1996; Saurin et al. 1997; Ward et al. 2001; Weihrauch et al. 2000; Wong et al. 2002, 2003). Based on this information, vinyl chloride is considered to be a carcinogen in humans by both IARC and EPA (IARC 1987; IRIS 2005). It has been suggested that inhalation exposure to vinyl chloride in humans may also result in increased incidences of cancers of the brain and central nervous system, respiratory tract, connective and other soft tissues, and lymphatic/hematopoeitic systems (for additional detail, see ATSDR 2004b); 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 (Adkins et al. 1986; Bi et al. 1985; Drew et al. 1983; Froment et al. 1994; Lee et al. 1977, 1978; Maltoni et al. 1981; Suzuki 1983).

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-chloro-acetaldehyde. The intermediary metabolites bind to macromolecules in the body. 2-Chloroethylene oxide is believed to bind primarily to DNA and RNA, whereas 2-chloroacetaldehyde binds primarily to proteins (Bolt et al. 1986; Guengerich and Watanabe 1979; Guengerich et al. 1979, 1981; Kappus et al. 1976; Watanabe et al. 1978a, 1978b). 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 (Akasaka et al. 1997; Chiang et al. 1997; Dosanjh et al. 1994; Guichard et al. 1996; Matsuda et al. 1995; Pandya and Moriya 1996; Zhang et al. 1995; Zielinski and Hergenhahn 2001). Four primary cyclic DNA etheno-adducts are formed by the reactive metabolites of vinyl chloride (1,N^6-ethenoadenine, 3,N^4-ethenocytosine, $N^{2,3}$-ethenoguanine, and 1,N^2-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 (Albertini et al. 2003, Barbin 1998, 2000; Kielhorn et al. 2000; Whysner et al. 1996). DNA crosslinks can also be formed because chloracetaldehyde 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.

D.4 Health Guidelines

ATSDR (2004b) derived an acute 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 gestational days 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 human equivalent concentration (HEC) as described in EPA guidelines (EPA 1994). Since the partition coefficient in mice is greater than that in humans, a default

value of 1 was used for the ratio and the duration-adjusted animal NOAEL (15 ppm) was equivalent to the $NOAEL_{HEC}$ (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 $NOAEL_{HEC}$ to derive the MRL of 0.5 ppm.

An intermediate-duration inhalation MRL of 0.03 ppm was derived for vinyl chloride, based on a lower 95% confidence limit (LEC_{10}) value of 5 ppm for hepatic centrilobular hypertrophy in rats (Thornton et al. 2002). All dichotomous models in the Benchmark Dose Software (BMDS version 1.3.2) were fit to the incidence data for centrilobular hypertrophy in the rats exposed to vinyl chloride by inhalation (Thornton et al. 2002). The LEC_{10} of a 10% extra risk (LEC) for hepatic centrilobular hypertrophy was selected as the benchmark response for the point of departure. Several models provided equivalent goodness-of-fit statistics. Therefore, the LEC_{10} value of 3 ppm, derived from the simplest model (Weibull), was selected as the point of departure for calculating an intermediate-duration inhalation MRL. The LEC_{10} of 3 ppm was duration-adjusted from intermittent (6 hours/day) to continuous exposure (3 ppm x 6/24 = 0.8 ppm). Following EPA (1994) methodology, the human equivalent concentration (LEC_{10HEC}) for an extrarespiratory effect produced by a category 3 gas was calculated by multiplying the duration-adjusted animal LEC_{10} by the ratio of the blood:gas partition coefficients in animals and humans $[(H_{b/g})A / H_{b/g})H]$. Since the partition coefficient in mice is greater than that in humans, a default value of 1 was used for the ratio and the duration-adjusted animal LEC_{10} (0.8 ppm) was equivalent to the LEC_{10HEC} (0.8 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 LEC_{10HEC} to derive the MRL of 0.03 ppm.

ATSDR (2004b) did not derive a chronic inhalation MRL for vinyl chloride because of the absence of a suitable LOAEL or NOAEL for derivation.

No acute- or intermediate-duration oral MRLs were derived for vinyl chloride because of an absence of data on the effects of oral exposure to vinyl chloride for these duration categories (ATSDR 2004b).

ATSDR (2004b) derived a chronic 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 the PBPK model used to derive EPA's RfD (Clewell et al. 2001; IRIS 2005). 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 of 0.09 mg/kg/day. Therefore, the human equivalent dose 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 human equivalent NOAEL.

EPA (IRIS 2005) 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 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 (IRIS 2005) derived a chronic RfC of 0.1 mg/m^3 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.

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* (IRIS 2005). EPA's current weight-of-evidence 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 (IRIS 2005). 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 $\mu g/m^3$ for continuous lifetime exposure from birth was estimated by EPA (IRIS 2005) 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 $\mu g/m^3$ for continuous lifetime exposure during adulthood was also estimated by EPA (IRIS 2005). An oral slope factor for continuous lifetime exposure from birth was estimated by EPA (IRIS 2005) to be 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 by EPA (IRIS 2005).

IARC (1987) lists vinyl chloride in Group 1 (*carcinogenic to humans*) based on sufficient evidence of carcinogenicity in humans and animals. NTP's Eleventh Report on Carcinogens (NTP 2005) reports that

vinyl chloride is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans.

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

The endpoints of concern for vinyl chloride in this mixture are hepatic, renal, immunological, and developmental. TTDS are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2004a). The derivations are based primarily on data provided in ATSDR (2004b), and in particular, the LSE tables.

Inhalation TTDs

Following EPA (1994) methodology, the human equivalent concentration ($NOAEL_{HEC}$) for an extrarespiratory effect produced by a category 3 gas, such as vinyl chloride, is calculated by multiplying the duration-adjusted animal NOAEL by the ratio of the blood:gas partition coefficients in animals and humans [(Hb/g)A / Hb/g)H]. Since the partition coefficients in rodents are greater than in humans (see ATSDR 2004b), a default value of 1 is used for the ratio.

Hepatic Effects, Intermediate Inhalation: The intermediate MRL for vinyl chloride is 0.03 ppm, based on hepatic effects.

Renal Effects, Intermediate Inhalation: Bi et al. (1985) identified a NOAEL of 10 ppm and a LOAEL of 100 ppm for increased kidney weights in rats exposed to vinyl chloride for 6 hours/day, 6 days/week for 12 months. This NOAEL, relevant to both intermediate and chronic duration exposure, was duration-adjusted to 2.1 ppm for continuous exposure, and a $NOAEL_{HEC}$ of 2.1 ppm was calculated as described previously under the heading Inhalation TTDs. Application of an uncertainty factor of 30 (3 for animal to human extrapolation using a dosimetric adjustment and 10 for intrahuman variability) yields a TTD_{RENAL} of 0.07 ppm.

Immunological Effects, Intermediate Inhalation: Bi et al. (1985) reported a LOAEL of 10 ppm for increased spleen weight in rats exposed to vinyl chloride for 6 hours/day, 6 days/week for 6 months. The LOAEL was duration-adjusted to 2.1 ppm for a continuous exposure scenario, and a $LOAEL_{HEC}$ of 2.1 was calculated as described previously under the heading Inhalation TTDs. Application of an uncertainty factor of 300 (3 for animal to human extrapolation using a dosimetric adjustment, 10 for intrahuman variability, and 10 for use of a LOAEL) would yield a TTD_{IMMUNO} of 0.007 ppm. However, this would fall below the MRL; the MRL of 0.03 ppm will be adopted as the TTD_{IMMUNO} for vinyl chloride.

Developmental Effects, Intermediate Inhalation: The acute 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 $NOAEL_{HEC}$) during organogenesis, and is adopted as the TTD_{DEVEL} for intermediate exposure.

Hepatic Effects, Chronic Inhalation: A $TTD_{HEPATIC}$ of 0.01 ppm was derived from the intermediate MRL; see explanation in Chapter 3.

Renal Effects, Chronic Inhalation: Bi et al. (1985) identified a NOAEL of 10 ppm and a LOAEL of 100 ppm for increased kidney weights in rats exposed to vinyl chloride for 6 hours/day, 6 days/week for 12 months. This NOAEL, relevant to both intermediate and chronic duration exposure, was duration-adjusted to 2.1 ppm for continuous exposure, and a $NOAEL_{HEC}$ of 2.1 ppm was calculated as described previously under the heading Inhalation TTDs. Application of an uncertainty factor of 30 (10 for animal to human extrapolations and 10 for intrahuman variability) yields a TTD_{RENAL} of 0.07 ppm.

Immunological Effects, Chronic Inhalation: A TTD_{IMMUNO} of 0.01 ppm was derived from the intermediate TTD for that endpoint; see explanation in Chapter 3.

Developmental Effects, Chronic Inhalation: A TTD_{DEVEL} of 0.2 ppm was derived from the intermediate 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 oral MRL of 0.003 mg/kg/day based on liver effects is adopted as a conservative value for intermediate exposure.

Renal Effects, Intermediate and Chronic Oral: No reports of renal effects following oral exposure to vinyl chloride were located.

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

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 Inhalation TTDs:

$MRL_{HEPATIC}$ = 0.03 ppm
TTD_{RENAL} = 0.07 ppm
TTD_{IMMUNO} = 0.03 ppm
TTD_{DEVEL} = 0.5 ppm

Chronic Inhalation TTDs:

$TTD_{HEPATIC}$ = 0.01 ppm
TTD_{RENAL} = 0.07 ppm
TTD_{IMMUNO} = 0.01 ppm
TTD_{DEVEL} = 0.2 ppm

Intermediate and Chronic Oral TTDs:

$MRL_{HEPATIC}$ = 0.003 mg/kg/day (chronic), adopted as $TTD_{HEPATIC}$ for intermediate
TTD_{RENAL} = Not derived, no data
TTD_{IMMUNO} = Not derived, no data
MRL_{DEVEL} = Not derived, no data

D.6 References

Adkins B Jr, Van Stee EW, Simmons JE, et al. 1986. Oncogenic response of strain A/J mice to inhaled chemicals. J Toxicol Environ Health 17:311–322.

Akasaka S, Langouet S, Fink S, et al. 1997. Site-specific mutagenesis with two vinyl chloride-derived DNA adducts and comparison to homologs. Proc Am Assoc Cancer Res 38:124.

Albertini R, Clewell H, Himmelstein MW, et al. 2003. The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride, and benzene. Regul Toxicol Pharmacol 37:105–132.

ATSDR 2004a. Guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

ATSDR. 2004b. Toxicological profile for vinyl chloride. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxpro2.html. October 14, 2005.

Barbin A. 1998. Formation of DNA etheno adducts in rodents and humans and their role in carcinogenesis. Acta Biochim Pol 45(1):145–161.

Barbin A. 2000. Etheno-adduct forming chemicals: from mutagenicity testing to tumor mutation spectra. Mutat Res 462:58–69.

Berk PD, Martin JF, Waggoner JG. 1975. Persistence of vinyl chloride induced liver injury after cessation of exposure. Ann NY Acad Sci 31:70–77.

Bi W, Wang Y, Huang M, et al. 1985. Effect of vinyl chloride on testis in rats. Ecotoxicol Environ Safety 10:281–289.

Black C, Pereira S, McWhirter A, et al. 1986. Genetic susceptibility to scleroderma-like syndrome in symptomatic and asymptomatic workers exposed to vinyl chloride. J Rheumatol 13:1059–1062.

Bolt HM, Filser JG, Laib RJ, et al. 1980. Binding kinetics of vinyl chloride and vinyl bromide at very low doses. In: Quantitative aspects of risk assessment in chemical carcinogenesis. Arch Toxicol Suppl 3:129–142.

Bolt HM, Kappus H, Buchter A, et al. 1976. Disposition of (1,2-^{14}C) vinyl chloride in the rat. Arch Toxicol 35:153–162.

Bolt HM, Laib RJ, Kappus H, et al. 1977. Pharmacokinetics of vinyl chloride in the rat. Toxicology 7:179–188.

Bolt HM, Laib RJ, Peter H, et al. 1986. DNA adducts of halogenated hydrocarbons. J Cancer Res Clin Oncol 112:92–96.

Buchter A, Bolt HM, Kappus H, et al. 1977. [Tissue distribution of 1,2-^{14}C–vinyl chloride in the rat.] Int Arch Occup Environ Health 39:27–32. (German)

Buchter A, Filser JG, Peter H, et al. 1980. Pharmacokinetics of vinyl chloride in the rhesus monkey. Toxicol Lett 6:33–36.

Byren D, Engholm G, Englund A, et al. 1976. Mortality and cancer morbidity in a group of Swedish VCM and PCV production workers. Environ Health Perspect 17:167–170.

Cheng T-J, Huang M-L, You N-C, et al. 1999. Abnormal liver function in workers exposed to low levels of ethylene dichloride and vinyl chloride monomer. J Occup Environ Med 41(12):1128–1133.

Chiang S-Y, Swenberg JA, Weisman WH, et al. 1997. Mutagenicity of vinyl chloride and its reactive metabolites, chloroethylene oxide and chloroacetaldehyde in a metabolically competent human β-lymphoblastois line. Carcinogenesis 18(1):31–36.

Clewell HJ, Gentry JM, Gearhart BC, et al. 1995. Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: examples with vinyl chloride and trichloroethylene. Chemosphere 31(1):2561-2578.

Clewell HJ, Gentry PR, Gearhart JM, et al. 2001. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. Sci Total Environ 274:37–66.

Creech JL, Johnson MN. 1974. Angiosarcoma of liver in the manufacture of polyvinyl chloride. J Occup Med 16:150–151.

Cullinan D, Johnson F, Grollman AP, et al. 1997. Solution structure of a DNA duplex containing the exocyclic lesion 3,N4-etheno-2'-deoxycytidine opposite 2'-deoxyguanosine. Biochemistry 36:11933–11943.

Dosanjh MK, Chenna A, Kim E, et al. 1994. All four known cyclic adducts formed in DNA by the vinyl chloride metabolite chloracetaldehyde are released by a human DNA glycosylase. Proc Natl Acad Sci USA 91:1024–1028.

Drew RT, Boorman GA, Haseman JK, et al. 1983. The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice, and hamsters. Toxicol Appl Pharmacol 68:120–130.

Du C-L, Wang J-D. 1998. Increased morbidity odds ratio of primary liver cancer and cirrhosis of the liver among vinyl chloride monomer workers. Occup Environ Med 55:528–532.

Edmonds LD, Anderson CE, Flynt JW, et al. 1978. Congenital central nervous system malformations and vinyl chloride monomer exposure: a community study. Teratology 17:137–142.

EPA. 1986. Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. Federal Register 51(185):33992–34003.

EPA. 1994. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency. EPA600890066F.

Falk H, Creech JL Jr, Heath CW Jr, et al. 1974. Hepatic disease among workers at a vinyl chloride polymerization plant. JAMA 230:59–63.

Feron VJ, Hendriksen CFM, Speek AJ, et al. 1981. Lifespan oral toxicity study of vinyl chloride in rats. Food Cosmet Toxicol 19:317–333.

Filser JG, Bolt HM. 1979. Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42:123–136.

Fortwengler P, Lewis RD, Reynolds L, et al. 1999. Empirical evidence that angiosarcoma of the liver caused by vinyl chloride exposure has a relatively high threshold dose in humans. Hepatology 30(4 Pt 2):504A.

Fox AJ, Collier PF. 1977. Mortality experience of workers exposed to vinyl chloride monomer in the manufacture of polyvinyl chloride in Great Britain. Br J Ind Med 34:1–10.

Froment O, Boivin S, Barbin A, et al. 1994. Mutagenesis of ras proto-oncogenes in rat liver tumors induced by vinyl chloride. Cancer Res 54(20):5340–5345.

Fučić A, Hitrec V, Garaj-Vrhovac V, et al. 1995. Relationship between locations of chromosome breaks induced by vinyl chloride monomer and lymphocytosis. Am J Ind Med 27(4):565–571.

Fučić A, Hitrec V, Garaj-Vrhovac V, et al. 1997. Clinical cytogenetics and toxogenetics with the common aim-risk assessment of chemical mutagens. Cytogenet Cell Genet 77(1-2):69.

Fučić A, Horvat D, Dimitrovic B. 1990. Localization of breaks induced by vinyl chloride in the human chromosomes of lymphocytes. Mutat Res 243:95–99.

Gedigke P, Muller R, Bechtelsheimer H. 1975. Morphology of liver damage among polyvinyl chloride production workers. A report on 51 cases. Ann N Y Acad Sci 246:278–285.

Grainger RG, Walker AE, Ward AM. 1980. Vinyl chloride monomer–induced disease: clinical, radiological, and immunological aspects. In: Preger L, ed. Induced disease, drug, irradiation, occupation. New York, NY: Grune and Stratton, 191–214.

Guengerich FP, Watanabe PG. 1979. Metabolism of ^{14}C- and ^{36}Cl-labeled vinyl chloride *in vivo* and *in vitro*. Biochem Pharmacol 28:589–596.

Guengerich FP, Crawford WM Jr, Watanabe PG. 1979. Activation of vinyl chloride to covalently bound metabolites: roles of 2-chloroethylene oxide and 2-chloroacetaldehyde. Biochemistry 18:5177–5182.

Guengerich FP, Mason PS, Stott WJ, et al. 1981. Roles of 2-haloethylene oxide and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in irreversible binding to protein and DNA. Cancer Res 41:4391–4398.

Guichard Y, Ghissassi FE, Nair J, et al. 1996. Formation and accumulation of DNA ethenobases in adult Sprague-Dawley rats exposed to vinyl chloride. Carcinogenesis 17(8):1553–1559.

Gwinner LM, Laib RJ, Filser JG, et al. 1983. Evidence of chloroethylene oxide being the reactive metabolite of vinyl chloride towards DNA: comparative studies with 2,2'-dichlorodiethyl ether. Carcinogenesis 4:1483–1486.

Heath CW Jr, Fable H, Creech JL Jr. 1975. Characteristics of cases of angiosarcoma of the liver among vinyl chloride workers in the United States. Ann N Y Acad Sci 246:231–236.

Hefner RE Jr, Watanabe PG, Gehring PJ. 1975. Preliminary studies of the fate of inhaled vinyl chloride monomer in rats. Ann N Y Acad Sci 246:135–148.

Ho SF, Phoon WH, Gan SL, et al. 1991. Persistent liver dysfunction among workers at a vinyl chloride monomer polymerization plant. J Soc Occup Med 41(1):10–16.

IARC. 1987. Vinyl chloride. IARC monographs on the evaluation of carcinogenic risks to humans. Supplement 7. International Agency for Research on Cancer. http://www.clc.iarc.fr/htdocs/monographs/suppl7/vinylchloride.html. June 06, 2004.

Infante PF, Wagoner JK, Waxweiler RJ. 1976. Carcinogenic, mutagenic, and teratogenic risks associated with vinyl chloride. Mutat Res 41:131–142.

IRIS. 2005. Integrated Risk Information System (IRIS). Cincinnati, OH: National Center for Environmental Assessment. http://www.epa.gov/iris/. October 14, 2005.

John JA, Smith FA, Leong BKJ, et al. 1977. The effects of maternally inhaled vinyl chloride on embryonal and fetal development in mice, rats and rabbits. Toxicol Appl Pharmacol 39:497–513.

John JA, Smith FA, Schwetz BA. 1981. Vinyl chloride: inhalation teratology study in mice, rats, and rabbits. Environ Health Perspect 41:171–177.

Jones DB, Smith PM. 1982. Progression of vinyl chloride induced hepatic fibrosis to angiosarcoma of the liver. Br J Ind Med 39:306–307.

Jones RD, Smith DM, Thomas PG. 1988. A mortality study of vinyl chloride monomer workers employed in the United Kingdom in 1940–1974. Scand J Work Environ Health 14:153–160.

Kappus H, Bolt HM, Buchter A, et al. 1976. Liver microsomal uptake of ^{14}C vinyl chloride and transformation to protein alkylating metabolites *in vitro*. Toxicol Appl Pharmacol 37:461–471.

Kielhorn JL, Melber C, Wahnschaffe U, et al. 2000. Vinyl chloride: still a cause for concern. Environ Health Perspect 108:579–588.

Krajewski J, Dobecki M, Gromiec J. 1980. Retention of vinyl chloride in the human lung. Br J Ind Med 37:373–374.

Laib RJ. 1982. Specific covalent binding and toxicity of aliphatic halogenated xenobiotics. Rev Drug Metabol Drug Interact 3:1–48.

Langauer-Lewowicka H, Dudziak Z, Byczkowska Z, et al. 1976. Cryoglobulinemia in Raynaud's phenomenon due to vinyl chloride. Int Arch Occup Environ Health 35:197–207.

Langauer-Lewowicka H, Kurzbauer H, Byczkowska Z, et al. 1983. Vinyl chloride disease – neurological disturbances. Int Arch Occup Environ Health 52:151–157.

Laplanche A, Clavel-Chapelon F, Contassot JC, et al. 1992. Exposure to vinyl chloride monomer: results of a cohort study after a 7 year follow-up. Br J Ind Med 49(2):134–137.

Lee CC, Bhandari JC, Winston JM, et al. 1977. Inhalation toxicity of vinyl chloride and vinylidene chloride. Environ Health Perspect 21:25–32.

Lee CC, Bhandari JC, Winston JM, et al. 1978. Carcinogenicity of vinyl chloride and vinylidene chloride. J Toxicol Environ Health 4:15–30.

Lee FL, Smith PM, Bennett B, et al. 1996. Occupationally related angiosarcoma of the liver in the United Kingdom 1972–1994. Gut 39:312–318.

Lelbach WK. 1996. A 25-year follow-up study of heavily exposed vinyl chloride workers in Germany. Am J Ind Med 29:446–458.

Lester D, Greenberg LA, Adams WR. 1963. Effects of single and repeated exposures of humans and rats to vinyl chloride. Am Ind Hyg Assoc J 24:265–275.

Lilis R, Anderson H, Nicholson WJ, et al. 1975. Prevalence of disease among vinyl chloride and polyvinyl chloride workers. Ann NY Acad Sci 246:22–41.

Liss GM, Greenberg RA, Tamburro CH. 1985. Use of serum bile acids in the identification of vinyl chloride hepatotoxicity. Am J Med 78:68–76.

Maltoni C, Lefemine G, Ciliberti A, et al. 1981. Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. Environ Health Perspect 41:3–29.

Marsteller HJ, Lelbach WK, Muller R, et al. 1975. Unusual splenomegalic liver disease as evidenced by peritoneoscopy and guided liver biopsy among polyvinyl chloride production workers. Ann NY Acad Sci 95–134.

Matsuda T, Yagi T, Kawanishi M, et al. 1995. Molecular analysis of mutations induced by 2-chloro-acetaldehyde, the ultimate carcinogenic form of vinyl chloride, in human cells using shuttle vectors. Carcinogenesis 16(10):2389–2394.

Mirkova E, Mihaylova A, Nosko M. 1978. [Embryotoxic and teratogenic effects in vinyl chloride]. Higiena i zdraveopazane 21:440–443. (Bulgarian)

Monson RR, Peters JM, Johnson MN. 1975. Proportional mortality among vinyl chloride workers. Environ Health Perspect 11:75–77.

NIOSH. 1977. A cross-sectional epidemiologic survey of vinyl chloride workers. Cincinnati, OH: U.S Department of Health and Human Services, National Institute for Occupational Safety and Health, Division of Surveillance, Hazard Evaluations, and Field Studies. PB274193.

NTP. 2005. Report on carcinogens. 11th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ntp-server.niehs.nih.gov/ntp/roc/toc11.html. October 14, 2005.

Ostlere LS, Harris D, Buckley C, et al. 1992. Atypical systemic-sclerosis following exposure to vinyl chloride monomer – a case report and review of the cutaneous aspects of vinyl chloride disease. Clin Exp Dermatol 17(3):208–210.

Pandya GA, Moriya M. 1996. 1,N6-ethenodeoxyadeosine, a DNA adduct highly mutagenic in mammalian cells. Biochemistry 35:11487–11492.

Patty FA, Yapt WP, Waite CP. 1930. Acute response of guinea pigs to vapors of some new commercial organic compounds. V. Vinyl chloride. Public Health Rep 45:1963–1971.

Pirastu R, Comba P, Reggiani A, et al. 1990. Mortality from liver disease among Italian vinyl chloride monomer/polyvinyl chloride manufacturers. Am J Ind Med 17:155–161.

Popper H, Thomas LB. 1975. Alterations of liver and spleen among workers exposed to vinyl chloride. Ann NY Acad Sci 246:172–194.

Reitz RH, Gargas ML, Anderson ME, et al. 1996. Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. Toxicol Appl Pharmacol 137:253–267.

Rinsky RA, Ott G, Ward E, et al. 1988. Study of mortality among chemical workers in the Kanawha Valley of West Virginia. Am J Ind Med 13:429–438.

Rosenman KD, Rizzo JE, Conomos MG, et al. 1989. Central nervous system malformations in relation to polyvinyl chloride production facilities. Arch Environ Health 44:279–282.

Sakabe H. 1975. Bone lesions among polyvinyl chloride production workers in Japan. Ann NY Acad Sci 246:78–79.

Sal'nikova LS, Kotsovskaya IA. 1980. [Effect of vinyl chloride on embryogenesis of rats.] Gig Tr Prof Zabol 46–47. (Russian)

Saurin J-C, Taniere P, Mion F, et al. 1997. Primary hepatocellular carcinoma in workers exposed to vinyl chloride. Cancer 79(9):1671–1677.

Simonato L, L'Abbe KA, Andersen A, et al. 1991. A collaborative study of cancer incidence and mortality among vinyl chloride workers. Scand J Work Environ Health 17(3):159–169.

Singer B. 1996. DNA damage: chemistry, repair, and mutagenic potential. Regul Toxicol Pharmacol 23:2–12.

Singer B, Spengler SJ, Chavez F, et al. 1987. The vinyl chloride-derived nucleoside, N2,3-etheno-guanosine, is a highly efficient mutagen in transcription. Carcinogenesis 8:745–747.

Spirtas R, McMichael AJ, Gamble J, et al. 1975. The association of vinyl chloride exposures with morbidity symptoms. Am Ind Hyg Assoc J 36:779–789.

Suciu I, Drejman I, Valeskai M. 1963. [Investigation of the diseases caused by vinyl chloride.] Medicina Interna 15:967–977. (Romanian)

Suciu I, Prodan L, Ilea E, et al. 1975. Clinical manifestations in vinyl chloride poisoning. Ann NY Acad Sci 246:53–69.

Suzuki Y. 1983. Neoplastic effect of vinyl chloride in mouse lung--lower doses and short-term exposure. Environ Res 32:91–103.

Tamburro CH, Makk L, Popper H. 1984. Early hepatic histologic alterations among chemical (vinyl monomer) workers. Hepatology 4:413–418.

Teta MJ, Schnatter AR, Ott MG, et al. 1990. Mortality surveillance in a large chemical company: the Union Carbide Corporation experience, 1974–1983. Am J Ind Med 17:435–447.

Theriault G, Allard P. 1981. Cancer mortality of a group of Canadian workers exposed to vinyl chloride monomer. J Occup Med 23:671–676.

Theriault G, Iturra H, Gingras S. 1983. Evaluation of the association between birth defects and exposure to ambient vinyl chloride. Teratology 27:359–370.

Thornton SR, Schroeder RE, Robison RL, et al. 2002. Embryo-fetal developmental and reproductive toxicology of vinyl chloride in rats. Toxicol Sci 68:207–219.

Til HP, Feron VJ, Immel HR. 1991. Lifetime (149-week) oral carcinogenicity study of vinyl chloride in rats. Food Chem Toxicol 29(10):713–718.

Til HP, Immel HR, Feron VJ. 1983. Lifespan oral carcinogenicity study of vinyl chloride in rats. Final report. Civo Institutes, TNO. Report No. V 93.285/291099.

Ungvary G, Hudak A, Tatrai E, et al. 1978. Effects of vinyl chloride exposure alone and in combination with trypan blue-applied systematically during all thirds of pregnancy on the fetuses of CFY rats. Toxicology 11:45–54.

Veltman G, Lange CE, Juhe S, et al. 1975. Clinical manifestations and course of vinyl chloride disease. Ann NY Acad Sci 246:6–17.

Vihko R, Vihko P, Maentausta O, et al. 1984. Assessment of early hepatotoxicity: biological monitoring and surveillance of workers exposed to chemicals. In: Aitio A, Riihimaki V, Vainio J, eds. Washington, DC: Hemosphere Publishing Co., 309–313.

Walker AE. 1976. Clinical aspects of vinyl chloride disease: skin. Proc R Soc Med 69:286–289.

Ward AM. 1976. Clinical aspects of vinyl chloride disease: evidence of an immune complex disorder in vinyl chloride workers. Proc R Soc Med 69:289–290.

Ward E, Boffetta P, Andersen A, et al. 2001. Update of the follow-up of mortality and cancer incidence among European workers employed in the vinyl chloride industry. Epidemiology 12:710–718.

Watanabe PG, Gehring PJ. 1976. Dose-dependent fate of vinyl chloride and its possible relationship to oncogenicity in rats. Environ Health Perspect 17:145–152.

Watanabe PG, McGowan GR, Gehring PJ. 1976a. Fate of ^{14}C vinyl chloride after single oral administration in rats. Toxicol Appl Pharmacol 36:339–352.

Watanabe PG, McGowan GR, Madrid EO, et al. 1976b. Fate of ^{14}C vinyl chloride following inhalation exposure in rats. Toxicol Appl Pharmacol 37:49–59.

Watanabe PG, Zempel JA, Gehring PJ. 1978a. Comparison of the fate of vinyl chloride following single and repeated exposure in rats. Toxicol Appl Pharmacol 44:391–399.

Watanabe PG, Zempel JA, Pegg DG, et al. 1978b. Hepatic macromolecular binding following exposure to vinyl chloride. Toxicol Appl Pharmacol 44:571–579.

Waxweiler RJ, Stringer W, Wagner JK, et al. 1976. Neoplastic risk among workers exposed to vinyl chloride. Ann NY Acad Sci 271:40–48.

Weber H, Reinl W, Greiser E. 1981. German investigations on morbidity and mortality of workers exposed to vinyl chloride. Environ Health Perspect 41:95–99.

Weihrauch M, Lehnert G, Kockerling F, et al. 2000. p53 mutation pattern in hepatocellular carcinoma in workers exposed to vinyl chloride. Cancer 88(5):1030–1036.

Whysner J, Conaway CC, Verma L, et al. 1996. Vinyl chloride mechanistic data and risk assessment: DNA reactivity and cross-species quantitative risk extrapolation. Pharmacol Ther 71(1/2):7–28.

Withey JR. 1976. Pharmacodynamics and uptake of vinyl chloride monomer administered by various routes to rats. J Toxicol Environ Health 1:381–394.

Wong O, Whorton MD, Foliart DE, et al. 1991. An industry-wide epidemiologic study of vinyl chloride workers, 1942–1982. Am J Ind Med 20(3):317–334.

Wong R-H, Chen P-C, Du C-l, et al. 2002. An increased standardized mortality ratio for liver cancer among polyvinyl chloride workers in Taiwan. Occup Environ Med 59:405–409.

Wong R-H, Chen P-C, Wang J-D, et al. 2003. Interaction of vinyl chloride monomer exposure and hepatitis B viral infection on liver cancer. J Occup Environ Med 45(4):379–383.

Wu W, Steenland K, Brown D, et al. 1989. Cohort and case-control analyses of workers exposed to vinyl chloride: an update. J Occup Med 31:518–523.

Zhang W, Rieger R, Iden C, et al. 1995. Synthesis of 3,N4-etheno, 3,N4-etheno and 3-(2-hydroxyetheyl) derivatives of 2'-deoxycytidine and their incorporation into oligomeric DNA. Chem Res Toxicol 8(1):148–156.

Zielinski B, Hergenhahn M. 2001. 2-Chloroacetaldehyde induces εdA DNA adducts in DNA of raji cells as demonstrated by an improved HPLC-fluorimetry method. Fresenius J Anal Chem 370(1):97–100.