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-dichloro-ethylene. 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 trichloro-ethylene 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 trichloro-ethylene 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 trichloro-ethylene. 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 trichloro-ethylene 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 tri-chloroethylene, 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-dichloro-ethylene, 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-di-chloroethylene'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-dichloro-ethylene. 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-di-chloroethylene 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-dichloro-ethylene 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 trichloro-ethylene. 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 tri-chloroethylene were observed in the studies. No studies were located in which pretreatment with 1,1-di-chloroethylene 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 tri-chloroethylene 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 tri-chloroethylene exposure was examined. Since the toxicological significance of the metabolic interaction can be inferred, and has been demonstrated for related binary mixtures (chloroform and trichloro-ethylene, 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 trichloro-

ethylene, 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 tri-

chloroethylene 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)