

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

2.1 Mixture of Concern

Toxicological data or PBPK models were not available for the complete mixture of concern or for any of the three- or four-component submixtures.

2.2 Binary Mixtures of Components

Toxicological data were available only for the binary mixture of carbon monoxide and methylene chloride; toxicological data on the other binary submixtures were not located. PBPK models for methylene chloride generally contain components describing the metabolism of methylene chloride to formaldehyde and carbon monoxide, but to date have not included estimations of co-exposure to either of these compounds.

In the following sections on the binary mixtures, the studies that focus on more relevant toxic endpoints are discussed first, with priority given to those conducted by simultaneous, longer-term inhalation exposure in mammals, followed by studies of less-relevant endpoints (e.g., acute lethal effects), and then studies of chemical interactions and of effects on tissue distribution or metabolism. At the end of each binary mixture section, the experimental results that may be used to support conclusions regarding joint toxic action are summarized in tables. For each listed endpoint and study, the tables present a conclusion regarding the direction of interaction for the influence of each chemical on the toxicity of the other. These conclusions include: additive (dose addition, response addition, or no apparent influence), greater than additive (synergism or potentiation), less than additive (antagonism, inhibition, or masking), or indeterminate (ambiguous, conflicting, or no data).

2.2.1 Carbon Monoxide and Formaldehyde

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of carbon monoxide and formaldehyde. No PBPK models for co-exposure to carbon monoxide and formaldehyde were located. From the available data, carbon monoxide and formaldehyde only share developmental effects as a shared target of toxicity. The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and formaldehyde.

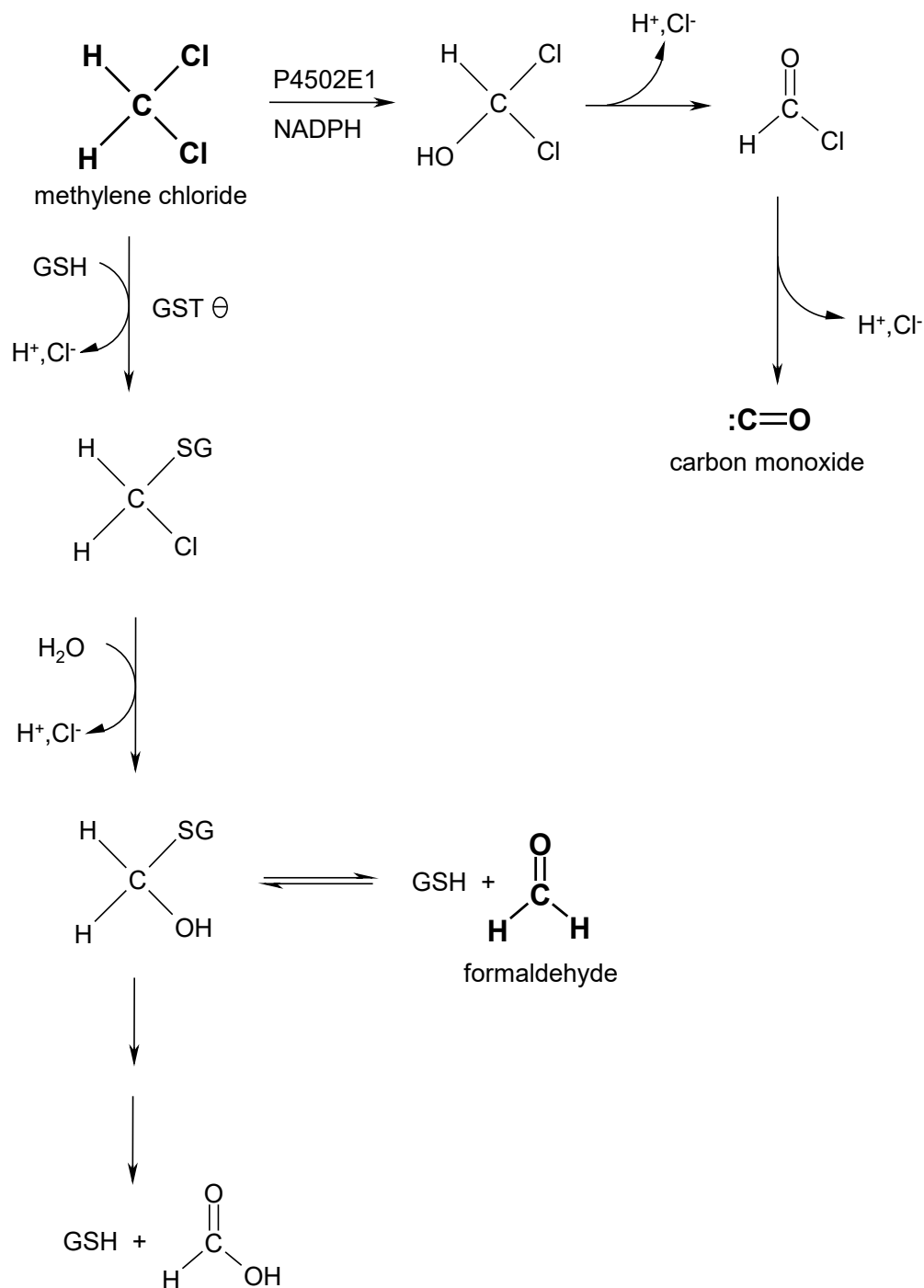
Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.2 Carbon Monoxide and Methylene Chloride

It is well-established that methylene chloride is metabolized by cytochrome P450 (CYP) isozyme 2E1 (CYP2E1) to carbon monoxide, primarily in the liver (see Appendix C and ATSDR 1999). The metabolism of methylene chloride is diagrammed in Figure 1. At low exposure levels, metabolism is primarily via the CYP pathway, resulting in carbon monoxide formation. Numerous studies have demonstrated the formation of COHb following exposure to methylene chloride in humans (Amsel et al. 2001; DiVincenzo and Kaplan 1981; Duenas et al. 2000; Fagin et al. 1980; Stevenson et al. 1978) and animals (DiVincenzo and Hamilton 1975; Kurppa et al. 1981; Rodkey and Collison 1977; Stevens et al. 1980). Available PBPK models for methylene chloride incorporate metabolism to carbon monoxide into the model structure; however, models to date have not incorporated the ability to evaluate co-exposures with carbon monoxide.

No studies examining the joint effects of co-exposure to carbon monoxide and methylene chloride in humans were located. Similarly, no studies of exposure of carbon monoxide prior to methylene chloride, or of methylene chloride exposure prior to carbon monoxide exposure, were located. An acute-duration study in humans comparing the neurological effects of methylene chloride with those of equivalent concentrations of carbon monoxide, in terms of blood COHb levels, found a more pronounced performance deficit for methylene chloride (Winneke 1981). Two distinct toxic actions were identified for methylene chloride neurological effects, nonspecific narcotic action (not associated with COHb) and COHb-induced hypoxia. Thus, the neurological effects of methylene chloride are only partially mediated by carbon monoxide formation, suggesting a potential for response addition for co-exposures of the two compounds for neurological effects mediated via elevated COHb levels. Winneke (1981) also suggested that there may be interaction between the two neurological mechanisms (narcotic, hypoxic) associated with methylene chloride; however, this hypothesis was not directly tested. The Winneke (1981) study is further limited by short duration (≤ 4.0 hours), evaluation of exposure levels much higher than environmental levels (e.g., ≥ 300 ppm for methylene chloride and ≥ 50 ppm for carbon monoxide), an unclear number of subjects per group, and a lack of quantitative presentation of results. These limitations decrease the usefulness of this study to evaluate potential interactions between these two compounds.

Figure 1. Metabolism of Methylene Chloride



GSH = glutathione; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; P4502E1 = cytochrome P450 enzyme involved in xenobiotic metabolism

Source: Ahmed et al. 1980

Kurppa et al. (1981) exposed groups of male Wistar rats to 100 ppm carbon monoxide, 1,000 ppm methylene chloride, or both for 3 hours. Exposure to carbon monoxide alone resulted in a mean 8.8% blood COHb and exposure to methylene chloride alone resulted in a mean 6.2% blood COHb. Combined exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride resulted in 14.6% blood COHb; thus, the simultaneous exposure is consistent with response-additive effects of blood COHb levels (see Table 3). No combined effects were noted on induction of CYP or ethoxycoumarin O-deethylase activities; effects on other endpoints were not reported. It is noteworthy that concentrations used in this study are much higher than those that are likely to be found in the environment or in homes. Other animal studies evaluating the effects of co-exposure to methylene chloride and carbon monoxide were not located. The Kurppa et al. (1981) study is limited by short duration (3 hours), evaluation of exposure levels much higher than environmental levels, and inclusion of only single concentrations for each compound either individually or in combination (precluding the evaluation of the dose-response nature of the potential interaction between these two compounds).

Table 3. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Methylene Chloride and Carbon Monoxide

Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no apparent influence	Less than additive		
Acute	Hematological		100 ppm carbon monoxide + 1,000 ppm methylene chloride (rats)		Response addition (rat blood COHb levels)	Kurppa et al. (1981)

COHb = carboxyhemoglobin

The metabolism of methylene chloride to carbon monoxide is complex, particularly when considering the possibility of co-exposure to carbon monoxide itself. Carbon monoxide absorption is driven by a concentration gradient, such that increased levels of blood COHb result in a saturation of absorption (see Appendix A); this would suggest that absorption of carbon monoxide following long-term exposure would be decreased during co-exposure to methylene chloride. However, methylene chloride interacts with the subunits of hemoglobin in a manner not completely understood, shifting both the oxygen and carbon monoxide association curves to the right, representing changes in affinity of these molecules for the heme iron (Harkey et al. 1979). Beyond the Kurppa et al. (1981) study, no studies were located that evaluated the effects of co-exposure to carbon monoxide and methylene chloride, so the potential impact of methylene chloride-derived carbon monoxide on the effects of inhaled carbon monoxide (and vice versa) has not been definitively evaluated.

2.2.3 Carbon Monoxide and Nitrogen Dioxide

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of carbon monoxide and nitrogen dioxide. No PBPK models for co-exposure to carbon monoxide and nitrogen dioxide were located. Several studies have reported simultaneous elevations of carbon monoxide and nitrogen dioxide in the home or in public environments (Cornforth et al. 1998; Lee et al. 1994); however, these studies have not evaluated the effects of exposure to these gases, individually or in combination, on human health. From the available data, carbon monoxide and nitrogen dioxide do not appear to have any sensitive shared targets of toxicity. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and nitrogen dioxide.

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

2.2.4 Carbon Monoxide and Tetrachloroethylene

No *in vivo* studies were located regarding possible joint toxic actions of carbon monoxide and tetrachloroethylene. One study demonstrated that oxidation of tetrachloroethylene by hydroxyl radicals (in a photochemical reaction chamber) may result in the formation of carbon monoxide (Itoh et al. 1994), but the extent to which this occurs *in vivo*, and the possible effects that it might have on the toxicities of carbon monoxide and/or tetrachloroethylene, has not been evaluated. The shared targets of toxicity for carbon monoxide and tetrachloroethylene include neurological effects, but studies evaluating the joint effects of the chemicals on either of these endpoints are not available. The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and tetrachloroethylene.

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

2.2.5 Formaldehyde and Methylene Chloride

As depicted in Figure 1 above and briefly described in Appendix C, a major metabolic pathway for methylene chloride involves conjugation to glutathione (GSH), catalyzed by glutathione S-transferase- θ (GST- θ). The resulting compound, chloromethyl-S-glutathione, can spontaneously react with water to form hydroxymethyl-S-glutathione, which can spontaneously degrade to formaldehyde and GSH or be

further metabolized to formate and GSH (see Figure 1). Numerous studies have suggested that the carcinogenic effects of methylene chloride noted in mice are the result of GST- θ -mediated metabolism to formaldehyde and subsequent interaction with cellular macromolecules, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Support for this hypothesis includes studies demonstrating the formation of DNA-protein crosslinks in mice, but not in hamsters, following acute-duration *in vivo* exposure to methylene chloride (Casanova et al. 1992), and demonstration that mammalian cells with higher levels of GST- θ exposed to methylene chloride generated larger numbers of DNA-protein crosslinks and RNA-formaldehyde adducts (Casanova et al. 1997). In human cells, only those cells that express the theta-glutathione-S-transferase (GSTT1-1) gene (the product of which is the GST- θ enzyme) generate DNA-protein crosslinks and RNA-formaldehyde adducts in response to methylene chloride exposure; levels in cells without the gene are not different from background (Casanova et al. 1997). Similarly, formaldehyde production was not detected in human erythrocytes (Hallier et al. 1994). El-Masri et al. (1999) developed PBPK models in humans and mice to evaluate the influence of the GSTT1-1 polymorphism on the risk of carcinogenesis from methylene chloride exposure.

While the metabolism of methylene chloride to formaldehyde is well-established, studies of the effect of co-exposure to formaldehyde and methylene chloride, either *in vivo* or *in vitro*, were not located. Numerous PBPK models exist that describe the metabolism of methylene chloride to formaldehyde; however, none of these models to date also incorporated simulations of the effects of co-exposure. Given that both formaldehyde and methylene chloride are believed to cause tumors by the reaction of formaldehyde with DNA and/or RNA, an additive association between the carcinogenicity of the two is likely. Since formaldehyde is the proximal toxicant, at the cellular level, this interaction would be dose additive. However, since the primary tissue targets for cancer differ between the compounds, at the organism level, the interaction would be response additive. Available mechanistic data are not sufficient to determine whether joint actions of methylene chloride and formaldehyde on respiratory effects will occur.

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

2.2.6 Formaldehyde and Nitrogen Dioxide

Marozienne and Grazuleviciene (2002) conducted an epidemiological study of residential air pollution. The only two pollutants measured were formaldehyde and nitrogen dioxide. Multivariate logistic regression was used to estimate the effect each separate pollutant would have on low birth weight and

premature birth. An increased risk of low birth weight was associated with formaldehyde exposure in the first trimester (odds ratio [OR] 2.20, 95% confidence interval [CI] 1.00–4.85) and prematurity was related to first trimester exposure to nitrogen dioxide (OR 1.67, 95% CI 1.28–2.18). When both chemicals were entered into the model together, the estimated effects did not change considerably except that the effect of exposure to nitrogen dioxide in the second trimester presented a risk of prematurity. The Maroziene and Grazuleviciene (2002) study is limited by the use of ecological monitoring data rather than personal exposure monitoring, the exclusion of other routinely monitored air pollutants (sulfur dioxide and total suspended particles) available in the ecological monitoring dataset from the analyses, and the lack of adjustment for other chemical exposures aside from smoking.

No *in vivo* or *in vitro* laboratory studies were located regarding possible joint toxic actions of formaldehyde and nitrogen dioxide. No PBPK models for co-exposure to formaldehyde and nitrogen dioxide were located. Formaldehyde and nitrogen dioxide share the respiratory system as a common site of toxicity, but studies evaluating the effects of exposure to both chemicals on respiratory endpoints are not available.

The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of formaldehyde and nitrogen dioxide.

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

2.2.7 Formaldehyde and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of formaldehyde and tetrachloroethylene. No PBPK models for co-exposure to formaldehyde and tetrachloroethylene were located. From the available data, formaldehyde and tetrachloroethylene do not appear to have any sensitive shared noncancer targets of toxicity. Both compounds have been shown to be tumorigenic; however, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of formaldehyde and tetrachloroethylene.

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

2.2.8 Methylene Chloride and Nitrogen Dioxide

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of methylene chloride and nitrogen dioxide. No PBPK models for co-exposure to methylene chloride and nitrogen dioxide were located. The available data indicate that methylene chloride and nitrogen dioxide are both capable of eliciting effects on the respiratory system, but no studies have evaluated the effect of co-exposure on respiratory endpoints. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of methylene chloride and nitrogen dioxide.

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

2.2.9 Methylene Chloride and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of methylene chloride and tetrachloroethylene. No PBPK models for co-exposure to methylene chloride and tetrachloroethylene were located. The available data indicate that methylene chloride and tetrachloroethylene are both capable of eliciting neurological and tumorigenic effects, but no studies have evaluated the effect of co-exposure on these endpoints. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of methylene chloride and tetrachloroethylene. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride, CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment.

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

2.2.10 Nitrogen Dioxide and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of nitrogen dioxide and tetrachloroethylene. No PBPK models for co-exposure to nitrogen dioxide and tetrachloroethylene were located. From the available data, nitrogen dioxide and tetrachloroethylene do not appear to have any sensitive shared targets of toxicity. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of nitrogen dioxide and tetrachloroethylene.

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

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

The carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene mixture is of concern because these chemicals, either alone or in combination, may be found in the home. The exposure route is primarily inhalation, and exposure durations are primarily intermediate to chronic. No epidemiological or toxicological studies of the complete mixture or for any of the three- or four-component submixtures are available. Similarly, no PBPK models are available for the complete mixture or for any of the three- or four-component submixtures. Some information and studies are available for binary mixtures of the components, but they are not adequate to support a quantitative assessment of interactions. Therefore, the WOE approach is appropriate (ATSDR 2001, 2018) to predict the potential impact of interactions (i.e., deviation from additivity). This approach involves determining, for each binary mixture, the WOE for the influence of one component on the toxicity of the other, and vice versa.

The binary weight-of-evidence (BINWOE) classification scheme is summarized in Table 4. 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, or lack thereof, 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, 2018). Rationales for the BINWOE determinations are presented in Tables 6–17 at the end of this section. The BINWOE determinations are presented for the binary mixtures in the same order as these mixtures were considered in Section 2.2.

Table 4. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

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

There are 10 unique binary pairs of chemicals in this mixture of 5 chemicals. For the following seven pairs of chemicals, no pertinent interaction data were found, and understanding of mechanisms of action is too incomplete to make projections of joint toxic actions:

- carbon monoxide and formaldehyde
- carbon monoxide and nitrogen dioxide
- carbon monoxide and tetrachloroethylene
- formaldehyde and tetrachloroethylene
- methylene chloride and nitrogen dioxide
- methylene chloride and tetrachloroethylene
- nitrogen dioxide and tetrachloroethylene

Evidence of varying quality and quantity is available supporting projections of additive joint toxic action for the following three pairs of chemicals (summarized in Table 5, with details provided in tables referenced below):

- carbon monoxide and methylene chloride (Tables 6 and 7)
- formaldehyde and methylene chloride (Tables 10 and 11)
- formaldehyde and nitrogen dioxide (Tables 12 and 13)

In summary, there are no data to suggest that non-additive interactions occur for any of the component pairs of the mixture, although it should be emphasized that studies designed to identify and characterize mode of joint toxic action of the components are, for the most part, unavailable.

Table 5. Matrix of BINWOE Determinations for Intermediate- or Chronic-Duration Simultaneous Exposure to Chemicals of Concern^a

		ON THE TOXICITY OF				
		Carbon monoxide	Formaldehyde	Methylene chloride	Nitrogen dioxide	Tetrachloro-ethylene
EFFECT OF	Carbon monoxide		?	IA2 h,n ? r,c	?	?
	Formaldehyde	?		IB1 c IIC1 r ? h,n	IIIC2 r	?
	Methylene chloride	IA2 h IIB2 n,d	IB1 r,c		?	?
	Nitrogen dioxide	?	IIIC2 r ? c	?		?
	Tetrachloroethylene	?	?	?	?	

^aThe BINWOE scheme was explained in Table 4 (ATSDR 2001, 2018). Data supporting the BINWOE determinations are presented in Tables 6–17; some BINWOE are based on results from high-level, acute-duration exposure studies. Additivity is likely at low-level exposures; dose additivity is assumed for noncancer effects and response additivity is assumed for carcinogenic effects.

BINWOE = binary weight-of-evidence; c = carcinogenic; d = developmental; h = hematological; n = neurological; r = respiratory

Table 6. Effect of Carbon Monoxide on Methylene Chloride

BINWOE: IA2 for hematological effects
BINWOE: IA2 for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The metabolism of methylene chloride to carbon monoxide is well-documented. Acute-duration rat data from Kurppa et al. (1981) indicate a response-additive effect of co-exposure on hematological endpoints. Similarly, Winneke (1981) suggested the potential for a response-additive effect of acute neurological effects for the two compounds in humans. Data suggesting possible interactions on respiratory effects, hepatic effects, or cancer are not available.

Mechanistic Understanding – The mechanism by which methylene chloride elicits effects on the hematological and nervous systems is believed to at least partially involve the metabolism of methylene chloride to carbon monoxide by CYP2E1. A human study comparing the acute neurological effects of methylene chloride with those of equivalent concentrations of carbon monoxide, in terms of blood COHb levels, found a more pronounced performance deficit for methylene chloride (Winneke 1981). Therefore, two distinct neurological mechanisms for methylene chloride were identified: nonspecific narcosis and COHb hypoxia. Based on this, the neurological effects of methylene chloride are partially mediated by carbon monoxide formation, suggesting the potential for a response-additive effect for co-exposures of the two compounds. The hematological effects of methylene chloride generally involve the formation of COHb; numerous studies in humans and animals have demonstrated the formation of COHb following methylene chloride exposure (see ATSDR 1999 and Appendix C). COHb-related effects are therefore expected to be additive, based on total blood COHb levels formed by the two compounds. This has been verified for acute-duration exposures by Kurppa et al. (1981), who found additive COHb levels following joint exposures to methylene chloride and carbon monoxide in rats. The mechanisms by which methylene chloride causes respiratory and carcinogenic effects have not been conclusively established, but as these effects have not generally been established as sensitive effects of carbon monoxide exposure, they are unlikely to be the result of metabolism of methylene chloride to carbon monoxide. A rating of I reflects the strong mechanistic understanding of the nature of the interaction.

Toxicological Significance – Only one study has directly evaluated the effects of co-exposure to carbon monoxide and methylene chloride. Kurppa et al. (1981) found that co-exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride for a single 3-hour period resulted in response addition of blood COHb formation in rats, based on measurements from single exposure to the chemicals (8.8% for CO, 6.2% for methylene chloride, and 14.6% for combined). Thus, for acute-duration exposures to mixtures of the two, COHb formation appears to be additive. Interaction data are not available for longer combined exposures to carbon monoxide and methylene chloride. A rating of A reflects that the significance of the interaction has been directly demonstrated.

Modifying Factors – The rating of “2” was used to reflect that available data on combined exposure were from an acute-duration exposure, rather than one of longer duration.

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

Table 7. Effect of Methylene Chloride on Carbon Monoxide

BINWOE: IA2 for hematological effects
BINWOE: IIB2 for neurological effects
BINWOE: IIB2 for developmental effects

Direction of Interaction – The metabolism of methylene chloride to carbon monoxide is well-documented. Acute-duration data from Kurppa et al. (1981) indicate a response-additive effect of co-exposure on COHb formation in rats, which is the primary mechanism of the toxic effects of carbon monoxide. It is therefore anticipated that for other sensitive effects of carbon monoxide, methylene chloride exposure will result in response addition, based on the formation of endogenous carbon monoxide.

Mechanistic Understanding – Carbon monoxide's toxicity is primarily the result of the formation of COHb and a resulting decrease in the oxygen-carrying capacity of the blood. Metabolism of methylene chloride to carbon monoxide by CYP, and the resulting formation of COHb, is well-documented (see Appendix C and ATSDR 1999). A study by Kurppa et al. (1981) demonstrated a response-additive effect of acute-duration co-exposure to methylene chloride and carbon monoxide on blood COHb formation in rats. While it therefore seems reasonable to assume a response-additive effect of co-exposure based on COHb formation, the effects of combined exposure have received only limited study and available PBPK models for methylene chloride have not been adapted to model co-exposure to the compounds. A rating of I reflects the strong mechanistic understanding of the nature of the interaction for hematological effects, while a rating of II reflects that the mechanism of interaction can be inferred for other effects.

Toxicological Significance – Only one study has directly evaluated the effects of co-exposure to methylene chloride and carbon monoxide. Kurppa et al. (1981) found that co-exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride for a single 3-hour period resulted in response addition of blood COHb formation in rats, based on measurements from single exposure to the chemicals (8.8% for CO, 6.2% for methylene chloride, and 14.6% for combined). Thus, for acute-duration exposures to mixtures of the two, COHb formation appears to be additive. Interaction data are not available for longer combined exposures to methylene chloride and carbon monoxide. A rating of A reflects that the significance of the interaction has been directly demonstrated for hematological effects, while a rating of B indicates that such interactions can be inferred but have not been demonstrated.

Modifying Factors – The rating of “2” was used to reflect that available data on combined exposure were from an acute-duration exposure, rather than one of longer duration.

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

Table 8. Effect of Carbon Monoxide on Tetrachloroethylene

BINWOE: ? for neurological effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with carbon monoxide will influence tetrachloroethylene toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between carbon monoxide and tetrachloroethylene.

Mechanistic Understanding – The primary shared target of toxicity for carbon monoxide and tetrachloroethylene is effects on the neurological system. Both compounds have been shown to cause neurological effects following high-dose exposures. However, the mechanisms by which tetrachloroethylene causes neurological effects have not been fully established but likely involve disruption of neuronal membrane structure and function. Therefore, available information is not sufficient to predict whether increased tissue hypoxia, the putative mechanism by which carbon monoxide-induced effects are produced, would have an impact on tetrachloroethylene-induced neurological effects. Similarly, it is not known whether carbon monoxide could influence other endpoints affected by tetrachloroethylene, namely hepatic effects, or cancer.

Toxicological Significance – Relevant interaction data on pertinent health effects with simultaneous inhalation exposure were not located. No studies were located in which pretreatment with carbon monoxide prior to tetrachloroethylene exposure was examined.

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

Table 9. Effect of Tetrachloroethylene on Carbon Monoxide

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for developmental effects

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with tetrachloroethylene will influence carbon monoxide toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between tetrachloroethylene and carbon monoxide.

Mechanistic Understanding – The primary shared target of toxicity for tetrachloroethylene and carbon monoxide is effects on the neurological system. Both compounds have been shown to cause neurotoxicity following high-dose exposures. However, the mechanisms by which tetrachloroethylene causes neurotoxicity has not been fully established but likely involve disruption of neuronal membrane structure and function. Therefore, available information is not sufficient to predict whether increased tissue hypoxia, the putative mechanism by which carbon monoxide-induced effects are produced, would be influenced by tetrachloroethylene-induced neurotoxicity. Similarly, it is not known whether tetrachloroethylene could influence other sensitive targets of toxicity affected by carbon monoxide, namely hematological effects or developmental effects.

Toxicological Significance – Relevant interaction data on pertinent health effects with simultaneous inhalation exposure were not located. No studies were located in which pretreatment with tetrachloroethylene prior to carbon monoxide exposure was examined.

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

Table 10. Effect of Formaldehyde on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: IIC for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: IB for cancer

Direction of Interaction – Studies have strongly implicated the formation of formaldehyde in the carcinogenic effects of methylene chloride (Graves and Green 1996; Graves et al. 1994a, 1994b, 1995, 1996); the direction of interaction is therefore expected to be additive. Similarly, metabolism to formaldehyde could partially explain the respiratory effects seen following high levels of methylene chloride exposure, implicating a dose-additive effect of combined exposure. However, other effects of methylene chloride (e.g., hematological, neurological, and hepatic effects) are not believed to be the result of metabolism to formaldehyde. The direction of interaction for these effects cannot be determined from available data.

Mechanistic Understanding – The metabolism of methylene chloride to formaldehyde has been well-established (see Appendix C and ATSDR 1999). The carcinogenic effects of methylene chloride are believed to be the result of metabolism to formaldehyde and subsequent nucleophilic attack of DNA (Graves and Green 1996; Graves et al. 1994a, 1994b, 1995, 1996). As such, additional exposure to formaldehyde, at the cellular level, is likely to result in additive joint toxicity. This is reflected by a rating of “I” for cancer. High-dose exposures to methylene chloride produce pulmonary effects (see Appendix C). The mechanism(s) for respiratory effects have not been fully elucidated (see Appendix C); however, metabolism to formaldehyde may contribute to observed effects. Since the association between respiratory effects and formaldehyde formation is not as strong as the association between carcinogenesis and formaldehyde formation following methylene chloride exposure, a rating of “II” for respiratory effects was assigned. The hematological and neurological effects of methylene chloride are believed to be at least partially the result of CYP-mediated metabolism to carbon monoxide, and as such are not likely to be appreciably affected by co-exposure to formaldehyde.

Toxicological Significance – Studies evaluating combined exposure to formaldehyde and methylene chloride, or describing pretreatment with formaldehyde prior to methylene chloride exposure, were not located. The inference is stronger for cancer effects, and warranted a “B” rating, while for respiratory effects the significance is less clear, and received a “C.”

Additional Uncertainties – Formaldehyde primarily exerts its effects at the portal of entry (e.g., nasal tissues). Therefore, effects (including any potential interactions with other chemicals) at distant sites may occur only when the capacities for local metabolism and disposition of formaldehyde are exceeded.

Table 11. Effect of Methylene Chloride on Formaldehyde

BINWOE: IB for respiratory effects
BINWOE: IB for developmental effects
BINWOE: IB for cancer

Direction of Interaction – The metabolism of methylene chloride to formaldehyde is well-described in the literature (see Appendix C and ATSDR 1999). The formation of intracellular formaldehyde is expected to result in dose-additive effects when combined with exogenous formaldehyde exposure.

Mechanistic Understanding – The majority of the effects of formaldehyde are due to the reactive nature of the molecule. Respiratory irritation is the primary effect noted following inhalation exposure to formaldehyde; formaldehyde may be interacting with cellular membranes or entering the cells and reacting with intracellular molecules. At high concentrations, formaldehyde may also produce developmental effects. Metabolism of methylene chloride to formaldehyde would result in a higher concentration of intracellular formaldehyde, implying an additive joint toxicity. Similarly, formaldehyde's carcinogenic effects are believed to be the result of the reaction of formaldehyde with DNA and/or RNA (see Appendix B). Methylene chloride-derived formaldehyde has been shown to form similar products with DNA and RNA. Both of these possible interactions received a rating of I.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and formaldehyde, or describing pretreatment with methylene chloride prior to formaldehyde exposure, were not located. For both endpoints, the association can be strongly inferred but has not been directly demonstrated; ratings of "B" were assigned.

Additional Uncertainties – While methylene chloride is metabolized into formaldehyde, its toxic effects are primarily distal to the portal of entry, suggesting potentially different cellular targets between methylene chloride and formaldehyde. However, nasal metaplasia has been reported in rats following chronic exposure to methylene chloride (see Appendix C), supporting potential for interactions with long-term exposure. Additionally, formaldehyde effects (including any potential interactions with other chemicals) at distant sites may occur when the capacities for local metabolism and disposition of formaldehyde are exceeded.

Table 12. Effect of Formaldehyde on Nitrogen Dioxide**BINWOE: IIC2 for respiratory effects**

Direction of Interaction – Based on a mutually shared mechanism of respiratory irritation, dose-additive effects of co-exposure are predicted.

Mechanistic Understanding – Nitrogen dioxide's effects on the respiratory system are believed to be primarily due to irritation along the portal of entry, owing to the reactive nature of the compound. The respiratory effects of formaldehyde are similarly believed to be due to irritation along the portal of entry. Dose additivity resulting from mutual respiratory irritation therefore seems reasonable. However, data supporting this hypothesis are not available. A confidence rating of "III" was therefore assigned.

Toxicological Significance – Studies evaluating combined exposure to formaldehyde and nitrogen dioxide, or describing pretreatment with formaldehyde prior to nitrogen dioxide exposure, were not located. A rating of "C" reflecting an unclear significance was assigned.

Modifying Factor – Because the available studies have not evaluated longer-term exposures, a rating of "2" was used for different exposure duration.

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

Table 13. Effect of Nitrogen Dioxide on Formaldehyde

BINWOE: IIC2 for respiratory effects
BINWOE: ? for developmental effects
BINWOE: ? for cancer

Direction of Interaction – Based on a mutually shared mechanism of respiratory irritation, dose-additive effects of co-exposure to formaldehyde and nitrogen dioxide are predicted. Available data are inadequate to determine what effect, if any, co-exposure to nitrogen dioxide will have on the developmental or carcinogenic effects of formaldehyde.

Mechanistic Understanding – Formaldehyde's effects on the respiratory system are believed to be primarily due to irritation along the portal of entry, owing to the reactive nature of the compound. The respiratory effects of nitrogen dioxide are similarly believed to be due to irritation along the portal of entry. Dose additivity resulting from mutual respiratory irritation therefore seems reasonable. However, data supporting this hypothesis are not available. A confidence rating of "III" was therefore assigned. Current understanding of the mechanisms of nitrogen dioxide and the developmental and carcinogenic effects of formaldehyde is not sufficient to predict the direction or extent of possible interactions on developmental or carcinogenic endpoints.

Toxicological Significance – Studies evaluating combined exposure to nitrogen dioxide and formaldehyde, or describing pretreatment with nitrogen dioxide prior to formaldehyde exposure, were not located. A rating of "C" reflecting an unclear significance was assigned.

Modifying Factor – Because the available studies have not evaluated longer-term exposures, a rating of "2" was used for different exposure duration.

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

Table 14. Effect of Methylene Chloride on Nitrogen Dioxide**BINWOE: ? for respiratory effects**

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with methylene chloride will influence nitrogen dioxide toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between methylene chloride and nitrogen dioxide.

Mechanistic Understanding – The primary shared target of toxicity for methylene chloride and nitrogen dioxide is effects on the respiratory system. While the mechanism of nitrogen dioxide's respiratory effects is thought to involve a direct reaction with cells along the respiratory tract, the mechanism of respiratory effects of methylene chloride is more complex. Available data are not sufficient to indicate possible effects of methylene chloride on nitrogen dioxide-induced respiratory effects.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and nitrogen dioxide, or describing pretreatment with methylene chloride prior to nitrogen dioxide exposure, were not located.

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

Table 15. Effect of Nitrogen Dioxide on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with nitrogen dioxide will influence methylene chloride toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between nitrogen dioxide and methylene chloride.

Mechanistic Understanding – The primary shared target of toxicity for methylene chloride and nitrogen dioxide is effects on the respiratory system. While the mechanism of nitrogen dioxide's respiratory effects is thought to involve a direct reaction with cells along the respiratory tract, the mechanism of respiratory effects of methylene chloride is more complex. Available data are not sufficient to indicate possible effects of nitrogen dioxide on methylene chloride-induced respiratory effects. Similarly, mechanistic data are not sufficient to allow for predictions of the possible effects of co-exposure to nitrogen dioxide on the hematological, neurological, hepatic, or carcinogenic effects of methylene chloride.

Toxicological Significance – Studies evaluating combined exposure to nitrogen dioxide and methylene chloride, or describing pretreatment with nitrogen dioxide prior to methylene chloride exposure, were not located.

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

Table 16. Effect of Methylene Chloride on Tetrachloroethylene

BINWOE: ? for neurological effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with methylene chloride will influence tetrachloroethylene toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between methylene chloride and tetrachloroethylene.

Mechanistic Understanding – Sensitive shared targets of toxicity for methylene chloride and tetrachloroethylene include neurological and hepatic effects. However, the mechanisms by which each causes effects on neurological endpoints are not clearly understood and no prediction as to the direction or extent of possible interactions can be made. Similarly, mechanisms by which the two compounds result in hepatic changes are not completely understood but are thought to involve metabolism to reactive intermediates. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride and CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment. Understanding the mechanisms of tetrachloroethylene toxicity is not sufficient to allow for the prediction of possible effects of methylene chloride on other targets of tetrachloroethylene toxicity.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and tetrachloroethylene, or describing pretreatment with methylene chloride prior to tetrachloroethylene exposure, were not located.

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

Table 17. Effect of Tetrachloroethylene on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with tetrachloroethylene will influence methylene chloride toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between tetrachloroethylene and methylene chloride.

Mechanistic Understanding – Sensitive shared targets of toxicity for methylene chloride and tetrachloroethylene include neurological and hepatic effects. However, the mechanisms by which each causes effects on neurological endpoints are not clearly understood and no prediction as to the direction or extent of possible interactions can be made. Similarly, mechanisms by which the two compounds result in hepatic changes are not completely understood but are thought to involve metabolism to reactive intermediates. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride and CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment. Understanding the mechanisms of methylene chloride toxicity is not sufficient to allow for the prediction of possible effects of tetrachloroethylene on other targets of methylene chloride toxicity.

Toxicological Significance – Studies evaluating combined exposure to tetrachloroethylene and methylene chloride, or describing pretreatment with tetrachloroethylene prior to methylene chloride exposure, were not located.

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 five-component mixture, or for four- or three-component submixtures, are available. Similarly, PBPK models describing the behavior of the five-component mixture, or for four- or three-component submixtures, are not available. In the absence of direct interaction data, a component-based approach was utilized. However, data on the joint toxic action of the component pairs of the mixture are generally lacking, with only limited data available for the methylene chloride-carbon monoxide component pair and no adequate joint action data available for the remaining 9 of the 10 component pairs of the mixture. Data on the potential mechanistic interactions between the component pairs are limited but were located for two of the component pairs: formaldehyde with methylene chloride and formaldehyde with nitrogen dioxide.

For the individual components, intermediate- and chronic-duration inhalation MRLs are available for formaldehyde, methylene chloride, and tetrachloroethylene. MRLs for exposures to carbon monoxide or nitrogen dioxide have not been derived.