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

2.1 Mixture of Concern

No data were located regarding health or pharmacokinetic endpoints in humans or animals exposed to mixtures containing all four of the chemicals: 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene.

No physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models were found for quaternary mixtures of these chemicals.

2.2 Component Mixtures

No PBPK/PD models were found for ternary or binary mixtures of these chemicals. The following subsections present evaluations of health effects and pharmacokinetic data and discussions of mechanistic information pertinent to the joint toxic action of combinations of the components.

2.2.1 1,1,1-Trichloroethane, Trichloroethylene, and Tetrachloroethylene

Stacey (1989) studied the joint action of 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene on renal and hepatic endpoints in rats *in vivo* and in isolated rat hepatocytes *in vitro*. Groups of five to six male Sprague-Dawley rats were given doses of 1,1,1-trichloroethane (15 mmol/kg or 2,001 mg/kg), trichloroethylene (10 mmol/kg or 1,314 mg/kg), or tetrachloroethylene (15 mmol/kg or 2,487 mg/kg) alone by intraperitoneal injection. Preliminary experiments indicated that these doses were near (but below) thresholds for hepatotoxic effects. Similar groups were given these doses in a ternary mixture (15 mmol/kg 1,1,1-trichloroethane + 10 mmol/kg trichloroethylene + 15 mmol/kg tetrachloroethylene) or in binary mixtures (e.g., 15 mmol/kg 1,1,1-trichloroethane + 10 mmol/kg trichloroethylene). The control group received corn oil (0.6 mg/kg). Animals were sacrificed after 24 hours; livers were weighed and blood was collected for analysis of alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), and urea. Individually, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene did not produce any significant changes in these endpoints at the administered doses. Combined administration of all three agents produced a significant decrease in liver:body weight ratio and significant increases in blood levels

of ALT, SDH, and urea. Similar results were obtained with *in vivo* exposure to binary mixtures except that the magnitude of the changes was not as great as the changes produced by the ternary mixture.

In the *in vitro* experiments (Stacy 1989), freshly isolated rat hepatocytes were incubated for up to 3 hours in medium containing 1,1,1-trichloroethane (2 or 5 μL added to sealed 25 mL flasks), trichloroethylene (2 or 4 μL), or tetrachloroethylene (1 or 2 μL). These levels were chosen because they were below the threshold for induction of toxic effects based on preliminary experiments. Other hepatocytes were incubated with medium containing all three agents or binary mixtures at all possible combinations of the exposure levels used in the individual treatments (8 ternary combinations and 12 binary combinations). The media were sampled after 1, 2, and 3 hours of incubation and analyzed for several indices of toxicity (potassium ions, ALT, and lactate dehydrogenase) released from the hepatocytes. Incubation in the presence of 1,1,1-trichloroethane, trichloroethylene, or tetrachloroethylene alone caused no significant changes in the cytotoxicity indices compared with the control values. In contrast, leakage rates of potassium ions, ALT, and lactate dehydrogenase were significantly greater than control values for hepatocytes incubated for 3 hours with seven of the eight ternary combinations. The lowest level ternary combination (2 μL 1,1,1-trichloroethane + 2 μL trichloroethylene + 1 μL tetrachloroethylene) did not significantly change the cytotoxicity indices. Incubation for 3 hours to 5/12, 5/12, and 6/12 of the tested binary combinations significantly increased leakage rates for potassium ion, ALT, and lactate dehydrogenase, respectively, compared with control rates.

The results from the *in vivo* and *in vitro* studies consistently show that exposure to binary and ternary mixtures of the agents, at doses that were below individual thresholds, produced mild toxic hepatic or renal responses, and that the responses to the ternary mixtures were generally greater than responses to the binary mixtures. Although the individual doses were below individual threshold doses for hepatic or renal responses, the doses are much greater than any that might be expected to be associated with general population exposure to these compounds. For example, EPA (as cited in ATSDR 1997b) estimated that about 5% of the U.S. population using public drinking water is exposed to tetrachloroethylene levels above 0.5 ppb (0.014 μg/kg/day). Total tetrachloroethylene intakes by Canadians has been estimated at 1.2–2.7 μg/kg/day (ATSDR 1997b). In contrast, the dose of tetrachloroethylene given to rats in this study was >2 g/kg. Due to design limitations, the results of the study cannot discern whether the joint action of these agents at the high dose levels tested is additive, greater-than-additive, or less-than-additive. The study did not include dose levels of the individual agents that produced changes in the endpoints or, alternatively, include mixtures comprised of equal parts of the component agents at dose levels that additively equaled the applied individual-agent dose level. Without at least one effective dose level for

each agent, no indications of the individual dose-response relationships are given, and no conclusion can be drawn concerning their joint toxic action, except if the tested mixture includes equal doses of the components that add to the tested dose level of the individual agents. Unfortunately, Stacey's mixtures did not have the appropriate compositions to determine joint action of the agents as follows:

1 unit agent A alone; 1 unit agent B alone; ½ unit A + ½ unit B mixture, or

1 unit A alone; 1 unit B alone; 1 unit C alone; 1/3 unit A + 1/3 unit B + 1/3 unit C mixture.

Stacey (1989) concluded that the joint action of these chemicals "may best be described as additive," but acknowledged that "when the individual treatments have no effect, use of this term is not entirely appropriate." Stacey (1989) also proposed that the term "positive interaction has been generally used to indicate that the binary combinations demonstrate toxicity, while single chemicals do not and that ternary mixtures generally show toxicity greater than binary combinations." It should be noted, however, that this term, while consistent with the results, is not synonymous with the terms additive or greater-than-additive.

2.2.2 1,1,1-Trichloroethane and 1,1-Dichloroethane

No studies were located that examined health or pharmacokinetic endpoints after exposure to binary mixtures of 1,1,1-trichloroethane and 1,1-dichloroethane compared with exposure to the chemicals alone.

Acute exposures to high airborne concentrations of 1,1,1,-trichloroethane or 1,1-dichloroethane can produce central nervous system depression and anesthesia along with cardiac arrhythmias that may lead to ventricular fibrillation (see Appendices A and B, ATSDR 1990, 1995). 1,1,1-Trichloroethane-induced nervous system depression and cardiac arrhythmias are thought to be induced by the parent chemical and not by metabolites, and the cardiac arrhythmias are thought to involve parent-chemical sensitization of the heart to epinephrine-induced arrhythmias (ATSDR 1995). Although 1,1-dichloroethane has been studied less than 1,1,1-trichloroethane with respect to mechanisms of producing nervous system depression and cardiac arrhythmias (ATSDR 1990, 1995), it is likely to act by similar mechanisms to those of 1,1,1-trichloroethane, given that other small molecular weight halogenated hydrocarbons and other solvents produce similar effects, especially nervous system depression (ATSDR 1997b; Snyder and Andrews 1996). It is plausible that 1,1,1-trichloroethane and 1,1-dichloroethane may jointly act in an additive manner to depress the central nervous system and sensitize the heart to epinephrine, but no studies were located that were designed to test this hypothesis.

Unlike certain other chlorinated alkanes (such as carbon tetrachloride, chloroform, 1,1,2-trichloroethane, and 1,2-dichloroethane), 1,1,1-trichloroethane and 1,1-dichloroethane are not potent hepatotoxic, renotoxic, or carcinogenic agents, but some animal studies have found possible carcinogenic and noncarcinogenic effects in the liver and/or kidney following repeated high level exposure scenarios (ATSDR 1990, 1995). These effects are believed to be caused by reactive intermediates formed via cytochrome P-450 (CYP) catalysis. The low potencies of 1,1,1-trichloroethane and 1,1-dichloroethane to produce these effects have been associated with the limited degree to which they are metabolized.

The difference in hepatotoxic potency between 1,1,1-trichloroethane and its isomer, 1,1,2-trichloroethane, has been associated with differences in the degree to which the two isomers are metabolized, providing support for the general hypothesis that reactive products of metabolism (e.g., free radicals that are formed by reductive dechlorination catalyzed by CYP isozymes), and not parent chemicals, are responsible for the hepatotoxicity and carcinogenicity of chlorinated alkanes (Plaa 1986). In rats and mice dosed by gavage, urinary excretion of metabolites accounted for >70% of administered doses of the 1,1,2-isomer, whereas >85% of the 1,1,1-isomer was excreted unchanged in expired air (Mitoma et al. 1985).

The difference in hepatotoxic and carcinogenic potency between 1,1-dichloroethane and its isomer, 1,2-dichloroethane, appears to be associated with differences in metabolic disposition for the two isomers (McCall et al. 1983; Mitoma et al. 1985). 1,2-Dichloroethane can be conjugated to glutathione, leading to a reactive intermediate that is thought to be key to its relatively more toxic nature (McCall et al. 1983). The formation of reactive intermediates from conjugation of 1,1-dichloroethane with glutathione does not appear to occur. 1,1-Dichloroethane's low potency is also related to the limited degree to which it is metabolized. In mice given high doses (700 or 1,800 mg/kg), metabolism accounted for only 7 or 29% of the administered dose (Mitoma et al. 1985).

The mild hepatotoxic effects of 1,1,1-trichloroethane and 1,1-dichloroethane are believed to be caused by reactive metabolites formed by CYP catalysis. No studies were located that were designed to examine whether or not coexposure to 1,1,1-trichloroethane and 1,1-dichloroethane would influence each other's metabolic disposition. Competitive metabolic interactions (at CYP2B1/2or CYP2E1 active sites) between 1,1,1-trichloroethane and 1,1-dichloroethane are plausible. It does not appear likely, however, that metabolic inhibition at this site would lead to toxicologically significant shunting to alternative pathways (e.g., transformation of 1,1,1-trichloroethane to dechlorinated radical intermediates and acetylene; see Appendix A) given that neither chemical is extensively metabolized by the CYP oxidative pathway, which is the major pathway for each.

Results regarding the ability of 1,1,1-trichloroethane to induce it's own metabolic machinery or induce hepatic levels of CYP isozymes suggest that repeated inhalation or oral exposure to 1,1,1-trichloroethane is not likely to markedly alter CYP-mediated hepatic metabolism, especially at daily administered dose levels <500 mg/kg/day (see Appendix A). For example, in male rats exposed for up to 12 days to oral doses of 0, 0.1, 0.5, 5.0, or 10.0 g/kg/day 1,1,1-trichloroethane, only the two highest doses induced hepatic microsomal activities of CYP2E1 and CYP2B1/2 (Bruckner et al. 2000). Studies designed to examine whether induction of hepatic CYP isozymes would influence the toxicity of 1,1-dichloroethane were not located, although induction by phenobarbital has been demonstrated to increase rates of 1,1-dichloroethane metabolism in rat hepatic microsomes (McCall et al. 1983). It appears unlikely, however (given that 1,1,1-trichloroethane appears to be a weak inducer of CYP isozymes), that coexposure with 1,1,1-trichloroethane would lead to an increased capacity to transform 1,1-dichloroethane to putative toxic metabolites.

Studies designed to examine the ability of 1,1-dichloroethane to induce hepatic enzymes were not located. Even if it were known that 1,1-dichloroethane could induce hepatic CYP isozymes involved in 1,1,1-trichloroethane metabolism, enhancement of 1,1,1-trichloroethane metabolism to such a degree that hepatotoxicity or carcinogenicity would be enhanced is not expected. Downstream enzymes may prevent the elevation of hepatic concentrations of a proximate toxicant(s) and repair mechanisms may efficiently fix any damage to cellular macromolecules. Furthermore, results from studies in which rats have been pretreated with phenobarbital or ethanol to enhance hepatic metabolism of 1,1,1-trichloroethane have not shown consistent evidence that potentiation of 1,1,1-trichloroethane hepatotoxicity occurs (Carlson 1973; Cornish et al. 1973; see Appendix A).

In summary, it is plausible that 1,1,1-trichloroethane and 1,1-dichloroethane may act jointly in an additive manner to produce central nervous system depression and heart sensitization to epinephrine, but studies designed to test this hypothesis were not located. The mild hepatotoxic potency of both of these chemicals appears to be mediated by reactive metabolic intermediates, but it is unclear how or if these chemicals may influence each other's metabolism. In general, neither of these chemicals is extensively metabolized, and any mutual interference that they may have on each other's metabolism may have little influence on their toxicity. Additive joint toxic action to produce liver and kidney effects is plausible, but studies directly designed to test this hypothesis were not located.

2.2.3 1,1,1-Trichloroethane and Trichloroethylene

Acute exposures to high airborne concentrations or large oral doses of 1,1,1-trichloroethane or trichloroethylene can produce central nervous system depression and anesthesia, as well as cardiac arrhythmias that can lead to ventricular fibrillation (ATSDR 1995, 1997a). The nervous system depression by 1,1,1-trichloroethane is thought to be induced by the parent chemical (ATSDR 1995), whereas nervous system effects from trichloroethylene involve the parent chemical as well as the metabolite, trichloroethanol. In support of this hypothesis, Mikiskova and Mikiska (1966) reported that intraperitoneally administered trichloroethanol was 5–6 times more effective than trichloroethylene in altering electrophysiological variables associated with central nervous system depression in guinea pigs. The cardiac arrhythmias are thought to involve parent-chemical sensitization of the heart to epinephrine-induced arrhythmias (ATSDR 1995, 1997a). It is plausible that 1,1,1-trichloroethane and trichloroethylene may jointly act in an additive manner to depress the central nervous system and sensitize the heart to epinephrine. However, no studies were located that were designed to test this hypothesis, and it is unknown whether or not they may influence each other's metabolism.

Another toxicity target that is shared by 1,1,1-trichloroethane and trichloroethylene is the liver. Animal experiments indicate that both are mild hepatotoxic agents whose tissue damaging activity may be due to reactive metabolic intermediates formed via CYP isozyme catalysis (ATSDR 1995, 1997a). No clear evidence for 1,1,1-trichloroethane carcinogenicity was found in animal cancer bioassays, including an adequate inhalation bioassay with rats and mice and two oral studies with design limitations (ATSDR 1995), whereas cancer of the liver and lung in B6C3F1 mice and cancer of the kidney and testes in rats have been observed in bioassays involving high level chronic exposure to trichloroethylene (ATSDR 1997a). Any carcinogenic action that these chemicals might exert is expected to be due to reactive metabolites (ATSDR 1995, 1997a; Bull 2000; Green 2000; Lash et al. 2000).

Animal studies indicate that the rate of 1,1,1-trichloroethane metabolism is much lower than that of trichloroethylene, even under conditions (e.g., repeated ethanol exposure) that induce hepatic CYP isozyme levels (Kaneko et al. 1994). In these studies, it was shown that ethanol pretreatment of rats increased the rate of metabolism of inhaled 1,1,1-trichloroethane to trichloroethanol and trichloroacetic acid, but most of the chemical still was expected to be eliminated (exhaled) unmetabolized (Kaneko et al. 1994). In contrast, ethanol pretreatment did not enhance trichloroethylene transformation to trichloroacetic acid or trichloroethanol after low-level trichloroethylene exposure (50–100 ppm), but did increase the rate of appearance of these metabolites in urine after exposure to high levels (500 or 1,000 ppm) of trichloro-

ethylene (Kaneko et al. 1994). Although Lee et al. (2000) recently reported that moderate to high (432 or 1,000 mg/kg), but not low (8 mg/kg), acute doses of trichloroethylene induced hepatic CYP2E1 activities in rats, results from studies of phenobarbital induction (Carlson 1973; Cornish et al. 1973) do not provide consistent evidence that CYP induction will lead to enhancement of 1,1,1-trichloroethane hepatotoxicity. Thus, trichloroethylene enhancement of 1,1,1-trichloroethane hepatoxicity is not expected, especially at administered trichloroethylene dose levels below 400 mg/kg when no CYP induction is expected.

Phenobarbital pretreatment and induction of hepatic CYP isozymes are associated with enhancement of acute high-level trichloroethylene hepatotoxicity in rodents (Allemand et al. 1978; Carlson 1973; Moslen et al. 1977; Nakajima et al. 1990), but no studies were located indicating that 1,1,1-trichloroethane pretreatment or coexposure with trichloroethylene would enhance trichloroethylene metabolism. 1,1,1-Trichloroethane modestly induced hepatic CYP 2B1/2 and 2E1 isozymes (involved in the bioactivation of trichloroethylene) only at near-lethal levels of exposure in rats (Bruckner et al. 2000; see Appendix A); thus 1,1,1-trichloroethane is not expected to enhance trichloroethylene hepatotoxicity via CYP induction at environmentally relevant exposure levels. Competitive metabolic interactions between these chemicals at catalytic sites of CYP isozymes does not appear to be likely either. 1,1,1-Trichloroethane is likely to be a poor competitor of trichloroethylene as suggested by observations that the extent of trichloroethylene metabolism is much greater than that of 1,1,1-trichloroethane. Furthermore, alteration of trichloroethylene metabolism at CYP catalytic sites is likely to be physiologically important only at high concentrations/exposure levels when substrate concentrations are in excess of enzyme catalytic sites. For example, CYP induction only altered trichloroethylene metabolic rates in rats exposed to high (500 or 1,000 ppm), but not low (50–100 ppm), trichloroethylene levels (Kaneko et al. 1994).

When adult male rats were exposed by inhalation to a mixture of 1,1,1-trichloroethane (500 ppm) and trichloroethylene (200 ppm) for 6 hours/day for 4 consecutive days and sacrificed 18 hours after cessation of the last exposure period, no trichloroethylene was detected in blood, brain tissue, lung tissue, or perirenal fat (Vainio et al. 1978). In contrast, 1,1,1-trichloroethane was detected in these tissues, presumably due to its slower rate of metabolism. These results are consistent with the hypothesis that, under these conditions, 1,1,1-trichloroethane did not inhibit metabolic disposition of trichloroethylene, but are not conclusive since the study did not include rats exposed to trichloroethylene alone.

The only located study that examined possible toxicological interactions between trichloroethylene and 1,1,1-trichloroethane is a report of *in vitro* and *in vivo* rat studies (Stacey 1989). Incubation of isolated rat

hepatocytes for up to 3 hours in medium containing trichloroethylene (2 or 4 μL added to sealed 25 mL flasks) or 1,1,1-trichloroethane (2 or 5 μL) did not cause statistically significant changes in intracellular potassium concentrations or leakage of ALT or lactate dehydrogenase, compared with hepatocytes incubated in control medium. Incubation in medium containing both trichloroethylene and 1,1,1-trichloroethane at all combinations of the exposure levels noted above did not significantly affect these indices of hepatocellular damage, except at the higher level of trichloroethylene when decreased intracellular potassium concentrations and increased leakage of ALT or lactate dehydrogenase were found compared with controls (Stacey 1989). These results are consistent with a combined effect of the two chemicals on membrane integrity at the higher mixed exposure level of trichloroethylene. Intraperitoneal injection of rats with 10 mmol/kg trichloroethylene (1,314 mg/kg) alone or 15 mmol/kg 1,1,1-trichloroethane (2,001 mg/kg) alone did not produce, 24 hours after injection, significant kidney (serum urea levels) or liver damage (serum ALT and SDH, and liver-to-body weight ratio) compared with controls injected with corn oil. These doses were expected to be just below individual threshold doses for liver and kidney effects. Combined exposure significantly increased serum ALT and SDH and significantly decreased mean liver:body weight ratio compared with controls. The results from this study clearly show that exposure to a mixture of trichloroethylene and 1,1,1-trichloroethane, at dose levels below the individual hepatotoxic thresholds for these chemicals, can produce liver damage. However, as discussed in more detail in Section 2.2.1, one cannot discern from the results whether the joint action of trichloroethylene and 1,1,1-trichloroethane is additive, greater-than-additive, or less-than-additive, due to study design limitations.

In summary, it is plausible that 1,1,1-trichloroethane and trichloroethylene may jointly act in an additive manner to produce cardiac sensitization and toxic effects in the nervous system (CNS depression), the liver, and the kidney, but evidence in support of this hypothesis is weak due to the limited and ambiguous joint toxic action data on liver and kidney endpoints (Stacey 1989), a lack of joint action data on cardiac sensitization and nervous system endpoints, and a lack of data regarding how these chemicals may enhance or inhibit each other’s metabolism and general disposition in the body.

Table 1. Summary of Available Data on the Influence of 1,1,1-Trichloroethane on Toxicity/Carcinogenicity of Trichloroethylene and the Influence of Trichloroethylene on Toxicity/Carcinogenicity after 1,1,1-Trichloroethane after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal Exposure (mg/kg/day)[a]						
1,1,1-Trichloroethane Influence on Toxicity/Carcinogenicity of Trichloroethylene						
acute	serum ALT and SDH, liver-to-BW ratio		2,001 + 1,314 (r)[b]		Endpoints were increased with mixed exposure, but not by single components alone. Joint action is ambiguous due to study design limitation.	Stacey 1989
Trichloroethylene Influence on Toxicity/Carcinogenicity of 1,1,1-Trichloroethane						
acute	serum ALT and SDH, liver-to-BW ratio		1,314 + 2,001 (r)[b]		Endpoints were increased with mixed exposure, but not by single components alone. Joint action is ambiguous due to study design limitation.	Stacey 1989

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] Species code: r = rat

ALT = alanine aminotransferase; BW = body weight; SDH = sorbitol dehydrogenase

2.2.4 1,1,1-Trichloroethane and Tetrachloroethylene

1,1,1-Trichloroethane and tetrachloroethylene are both metabolized initially by CYP isozymes, with glutathione conjugation as a minor pathway for tetrachloroethylene (ATSDR 1995, 1997b). Exhalation of nonmetabolized (parent) chemical is expected to be the principal route of excretion for both chemicals in humans due to the low rates of metabolism and high volatility. Central nervous system depression, common to both, is thought to be produced by interaction of the parent chemicals with neuronal membrane components (ATSDR 1995, 1997b). Metabolites of these chemicals are thought to be responsible for the liver and kidney effects observed in studies of rodents exposed to either 1,1,1-trichloroethane or tetrachloroethylene, but the potential for either chemical to cause liver or kidney effects in humans is generally thought to be low due to limited human capacity to metabolize them.

Studies designed to examine possible metabolic interactions between 1,1,1-trichloroethane and tetrachloroethylene are restricted to a study of urinary metabolites in rats exposed by inhalation for 8 hours to about 350 ppm 1,1,1-trichloroethane, 100 ppm tetrachloroethylene, or a mixture of both at these concentrations (Koizumi et al.1982). The mean rate of urinary excretion of trichloroethanol during exposure to the mixture (75 μg/kg/hour ±40) was significantly less than the mean rate during exposure to 1,1,1-trichloroethane alone (179 μg/kg/hour ±39). Trichloroethanol was not detected in the urine during or after exposure to tetrachloroethylene alone. The results suggest that tetrachloroethylene inhibited the metabolism of 1,1,1-trichloroethane. In general however, neither of these chemicals is extensively metabolized; any alterations that they may have on each other's metabolism should have little influence on their toxicity. Pretreatment of rats with CYP inducers, including ethanol (Cornish and Adefuin 1966; Klaassen and Plaa 1966), phenobarbital (Cornish et al. 1973; Moslen et al. 1977), or Aroclor 1254 (Moslen et al. 1977) has not consistently potentiated acute high level tetrachloroethylene hepatotoxicity. Likewise, results from similar studies with 1,1,1-trichloroethane do not provide consistent evidence of potentiation of acute hepatotoxicity from CYP induction (Carlson 1973; Cornish et al. 1973).

Results from studies of hepatic and renal endpoints in rats exposed by intraperitoneal injection and in isolated rat hepatocytes *in vitro* (Stacey 1989) are consistent with the hypothesis that 1,1,1-trichloroethane and tetrachloroethylene may jointly act in a positive manner, but cannot exclude the possibility of greater-than-additive or less-than-additive interactions due to study design limitations as discussed in Section 2.2.1. Incubation of isolated rat hepatocytes for up to 3 hours in medium containing tetrachloroethylene (1 or 2 μL added to sealed 25 mL flasks) or 1,1,1-trichloroethane (2 or 5 μL) did not cause statistically significant changes in intracellular potassium concentrations or leakage of ALT or lactate

dehydrogenase, compared with hepatocytes incubated in control medium. Incubation in medium containing both tetrachloroethylene and 1,1,1-trichloroethane at combinations of the exposure levels noted above did not significantly affect these indices of hepatocellular damage, except at the higher level of tetrachloroethylene when decreased intracellular potassium concentrations and increased leakage of ALT or lactate dehydrogenase were found (Stacey 1989). These results are consistent with a joint toxic effect on membrane integrity at the higher mixed exposure level of tetrachloroethylene. Intraperitoneal injection of rats with near-threshold doses of 15 mmol/kg tetrachloroethylene (2,487 mg/kg) or 15 mmol/kg 1,1,1-trichloroethane (2,001 mg/kg) alone did not significantly change, 24 hours after injection, measures of liver (serum ALT and SDH, and liver-to-body weight ratio) or kidney damage (serum urea levels) compared with controls injected with corn oil. However, coadministration of 15 mmol/kg tetrachloroethylene + 15 mmol/kg 1,1,1-trichloroethane caused statistically significant increases (compared with controls or either chemical alone) in serum ALT and SDH, a nonsignificant increase in serum urea, and a nonsignificant decrease in liver:body weight ratio. These results clearly show that a mixture of subthreshold doses of tetrachloroethylene and 1,1,1-trichloroethane can produce liver and/or kidney damage, but cannot clearly discern the mode of joint action due to study design limitations.

In summary, it is plausible that 1,1,1-trichloroethane and tetrachloroethylene may jointly act in an additive manner to produce cardiac sensitization and effects in the nervous system, the liver, and the kidney, but evidence in support of this hypothesis is weak due to the limited and ambiguous interaction data on liver and kidney endpoints (Stacey 1989), a lack of data regarding how these chemicals may enhance or inhibit each other's metabolism and general disposition in the body, and a lack of interaction data on cardiac sensitization and nervous system endpoints. There are data to suggest that tetrachloroethylene may suppress 1,1,1-trichloroethane metabolism in rats (Koizumi et al. 1982), but the limited degree to which 1,1,1-trichloroethane is metabolized suggests that this should have little effect on its toxicity.

Table 2. Summary of Available Data on the Influence of 1,1,1-Trichloroethane on Toxicity/Carcinogenicity of Tetrachloroethylene and the Influence of Tetrachloroethylene on Toxicity/Carcinogenicity of 1,1,1-Trichloroethane after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Intraperitoneal Exposure (mg/kg/day)[a]						
1,1,1-Trichloroethane Influence on Toxicity/Carcinogenicity of Tetrachloroethylene						
acute	serum ALT and SDH, liver-to-BW ratio		2,001 + 2,487 (r)[b]		Endpoints were changed with mixed exposure, but not by single components alone. Joint action is ambiguous due to study design limitation.	Stacey 1989
Tetrachloroethylene Influence on Toxicity/Carcinogenicity of 1,1,1-Trichloroethane						
acute	serum ALT and SDH, liver-to-BW ratio		2,487 + 2,001 (r)[b]		Endpoints were changed with mixed exposure, but not by single components alone. Joint action is ambiguous due to study design limitation.	Stacey 1989

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] Species code: r = rat

ALT = alanine aminotransferase; BW = body weight; SDH = sorbitol dehydrogenase

2.2.5 1,1-Dichloroethane and Trichloroethylene

No studies were located that examined health or pharmacokinetic endpoints after exposure to binary mixtures of 1,1-dichloroethane and trichloroethylene compared with exposure to the chemicals alone.

The initial steps in the metabolism of 1,1-dichloroethane and trichloroethylene involve oxidation catalyzed by CYP isozymes (ATSDR 1990, 1997a). The capacity of rodent hepatic liver microsomes to metabolize these chemicals has been demonstrated to be induced by phenobarbital and chronic ethanol pretreatment (Colacci et al. 1985; Cornish and Adefuin 1966; McCall et al. 1983; Nakajima et al. 1990, 1993; Sato et al. 1980, 1981), indicating that similar CYP isozymes are involved in metabolism of each (ATSDR 1990, 1997a). Competitive metabolic interactions at CYP catalytic sites between these chemicals are possible, but not likely. Although studies designed to test this hypothesis were not located, 1,1-dichloroethane is much more slowly metabolized than trichloroethylene (see below) and is, thus, unlikely to be an effective competitive inhibitor of trichloroethylene at CYP catalytic sites. Also, as discussed in Section 2.2.3, alteration of trichloroethylene metabolism at CYP catalytic sites is likely to be physiologically important only at high exposure levels (Kaneko et al. 1994). Given the limited extent to which 1,1-dichloroethane is metabolized, any influence that trichloroethylene may exert on 1,1-dichloroethane metabolism should have minimal influence on toxicity.

Like other solvents, both parent chemicals appear to sensitize the heart to epinephrine and to act on neuronal membranes producing reversible nervous system depression (ATSDR 1990, 1997a). Trichloroethanol, a metabolite of trichloroethylene, is thought to act similarly on neuronal membranes. It is plausible that both parent chemicals may act jointly in an additive manner to produce central nervous system depression and heart sensitization to epinephrine, but studies designed to test this hypothesis were not located.

In experiments with animals, trichloroethylene exposure has produced carcinogenic and noncarcinogenic responses in the liver and kidney that are thought to be caused by reactive metabolites (ATSDR 1997a). Studies designed to examine if coexposure to 1,1-dichloroethane would interfere with trichloroethylene metabolism and trichloroethylene induction of liver and kidney effects were not located. 1,1-Dichloroethane has a low potential for producing liver and kidney damage, as evidenced by the observation that 78-week oral exposure to 1,1-dichloroethane doses as high as 950 mg/kg/day in rats and 3,331 mg/kg/day in mice did not induce histological changes in liver or kidney tissue (ATSDR 1990; NCI 1977). 1,1-Dichloroethane's low hepatic and renal toxicities are associated with the limited extent to which it is

metabolized and the absence of formation of a reactive metabolite via conjugation with glutathione (such as that which is formed from 1,2-dichloroethane, the more toxic dichloroethane isomer) (McCall et al. 1983; Mitoma et al. 1985). For example, 48 hours after administration of high oral doses of 1,1-dichloroethane to rats (700 mg/kg) and mice (1,800 mg/kg), metabolism accounted for only about 7 and 29% of the administered doses, respectively (Mitoma et al. 1985). In contrast, metabolism accounted for about 30 and 82% of doses of trichloroethylene administered orally to rats (1,300 mg/kg) and mice (2,000 mg/kg) (Mitoma et al. 1985). Due to the limited extent to which it is metabolized, 1,1-dichloroethane is not likely to be an effective competitor of trichloroethylene metabolism and may not interfere with the toxic action of trichloroethylene. However, studies directly designed to test this hypothesis were not located.

In summary, despite limited mechanistic understanding of the toxic actions of 1,1-dichloroethane and trichloroethylene, it is plausible that they may act jointly in an additive manner to produce cardiac sensitization and effects on the nervous system, the liver, and the kidney, but studies directly designed to test hypotheses associated with this contention were not located.

2.2.6 1,1-Dichloroethane and Tetrachloroethylene

Studies that examined health or pharmacokinetic endpoints after exposure to binary mixtures of 1,1-dichloroethane and tetrachloroethylene compared with exposure to the chemicals alone were not located.

Like other solvents, both parent chemicals sensitize the heart to epinephrine and act on neuronal membranes producing reversible nervous system depression (ATSDR 1990, 1997b). It is plausible that 1,1-dichloroethane and tetrachloroethylene may act jointly in an additive manner to produce central nervous system depression and heart sensitization to epinephrine, but studies designed to test this hypothesis were not located.

Any carcinogenic or noncarcinogenic effects that these chemicals may exert on tissues outside of the nervous system (such as the liver and kidney) are expected to occur with repeated exposure to high doses (based on results from animal studies) and to be caused by reactive metabolites (ATSDR 1990, 1997b). The extent to which either is metabolized, however, is limited. For example, within 48 hours of administration of high oral doses, metabolism of 1,1-dichloroethane accounted for only about 7 and 29% of administered doses in rats (700 mg/kg) and mice (1,800 mg/kg), and metabolism of tetrachloroethylene accounted for only 5 and 22% of administered doses in rats (1,000 mg/kg) and mice (899 mg/kg)

(Mitoma et al. 1985). The initial steps in the metabolism of 1,1-dichloroethane and tetrachloroethylene involve oxidation catalyzed by CYP isozymes, indicating the potential for competitive metabolic interactions (ATSDR 1990, 1997b). However, neither of these chemicals is extensively metabolized, and any mutual interference that they may have on each other's metabolism may have little influence on their toxicity. Studies designed to examine possible metabolic interactions, however, were not located.

In summary, despite limited mechanistic understanding of the toxic actions of 1,1-dichloroethane and tetrachloroethylene, it is plausible that they may act jointly in an additive manner to produce cardiac sensitization and effects on the nervous system, the liver, and the kidney, but studies designed to test hypotheses associated with this contention were not located.

2.2.7 Trichloroethylene and Tetrachloroethylene

High levels of exposure to either trichloroethylene or tetrachloroethylene can produce reversible central nervous system depression due to parent chemical or metabolite (i.e., trichloroethanol) actions on neuronal membranes (ATSDR 1997a, 1997b). There is no conclusive evidence of liver damage, kidney damage, or cancer in humans exposed to either of these chemicals, but liver damage, kidney damage, and cancer have been demonstrated in bioassays of animals exposed to levels of trichloroethylene or tetrachloroethylene that are higher than those expected to be experienced by humans exposed in the workplace or in the general environment (ATSDR 1997a, 1997b). Although the mechanisms of liver damage, kidney damage, or cancer induction by these chemicals are incompletely understood, it is expected that metabolites, rather than parent chemicals, are responsible (ATSDR 1997a, 1997b; Green et al. 1990; Reitz et al. 1996). Metabolic pathways for the two chemicals, while not identical, share an initial epoxidation of the ethylene group catalyzed by CYP isozymes and a minor glutathione conjugation pathway, but trichloroethylene is metabolized to a greater extent than tetrachloroethylene (ATSDR 1997a, 1997b). For example, in rats pretreated with phenobarbital before intraperitoneal administration of 1,474 mg trichloroethylene/kg or 1,632 mg tetrachloroethylene/kg, rates of appearance of trichloroethylene metabolites in urine during 2-hour periods for up to 10 hours after injection were approximately 200- to 1,000-fold higher than rates for tetrachloroethylene metabolites (Ikeda and Imamura 1973). The principal mode of elimination of tetrachloroethylene from the body is exhalation of the parent chemical, whereas elimination of urinary metabolites is the more important route of elimination for trichloroethylene (ATSDR 1997a, 1997b). Due to overlap in CYP-mediated metabolic pathways for trichloroethylene and tetrachloroethylene, it is plausible that they may interfere with each other's metabolism. It is likely that such an interaction would have greater toxicologic significance for trichloroethylene (which is

extensively metabolized) than for tetrachloroethylene (which is poorly metabolized), and that it would occur only at high concentrations saturating CYP catalytic sites.

Seiji et al. (1989) reported that workers exposed to both trichloroethylene and tetrachloroethylene had lower levels of metabolites (total trichloro compounds) in the urine than workers exposed only to trichloroethylene at approximately the same level as in the mixed exposure. Geometric means of workplace air concentrations (determined from personal air samples) were 9.4 ppm trichloroethylene and 16.6 ppm tetrachloroethylene for the workers exposed to the mixture and 7.7 ppm trichloroethylene for workers exposed to trichloroethylene alone. In contrast, urinary levels of total trichloro compounds in workers exposed to tetrachloroethylene alone (at a geometric mean air concentration of 10.8 ppm) were much lower than levels in workers exposed to trichloroethylene alone or workers exposed to the mixture. The slope of a linear regression equation relating total trichloro compounds in urine (corrected for creatinine) to trichloroethylene air concentrations for workers exposed to trichloroethylene alone was 3.9-fold greater than the slope for a similar regression for workers exposed to the mixture. The slope of a regression relating total trichloro compounds in urine to tetrachloroethylene air concentrations for workers exposed to tetrachloroethylene alone was 5% of the slope of the regression relating trichloroethylene air concentrations to urinary total trichloro compounds in workers exposed to trichloroethylene alone. The data suggest that inhaled trichloroethylene is metabolized to a much greater extent than inhaled tetrachloroethylene, and that coexposure to tetrachloroethylene at fairly low exposure levels inhibits the metabolism of trichloroethylene in humans.

Stacey (1989) studied the joint action of trichloroethylene and tetrachloroethylene on renal and hepatic endpoints in rats *in vivo* and in isolated rat hepatocytes *in vitro*. Groups of five to six Sprague Dawley rats were given near-threshold doses of trichloroethylene (10 mmol/kg or 1,314 mg/kg) and tetrachloroethylene (15 mmol/kg or 2,487 mg/kg) alone and in combination, by intraperitoneal injection. The control group received corn oil (0.6 mg/kg). Animals were sacrificed after 24 hours; livers were weighed and blood was collected for analysis of ALT, SDH, and urea. Neither trichloroethylene nor tetrachloroethylene produced any effects individually at the doses administered. Combined administration produced a significant decrease in liver:body weight ratio, significant increases in ALT and SDH, and a nonsignificant increase in blood urea. The *in vitro* studies in isolated rat hepatocytes yielded similar results. Using doses at which the single chemicals did not produce effects (2 or 4 μL trichloroethylene, 1 or 2 μL tetrachloroethylene), mixtures containing either dose of trichloroethylene and tetrachloroethylene at the higher dose produced toxicity (leakage of potassium ions, ALT, and lactate dehydrogenase). These results clearly show that a mixture of subthreshold doses of tetrachloroethylene

and trichloroethylene can produce liver and/or kidney damage, but they cannot clearly discern the mode of joint action due to study design limitations as discussed in Section 2.2.1.

Goldsworthy and Popp (1987) investigated the joint effect of trichloroethylene and tetrachloroethylene on peroxisome proliferation in the livers and kidneys of rats and mice. Male Fischer 344 rats and B6C3F1 mice were given trichloroethylene alone (1,000 mg/kg/day), tetrachloroethylene alone (1,000 mg/kg/day) or both chemicals together (1,000 mg/kg/day trichloroethylene + 1,000 mg/kg/day tetrachloroethylene, one right after the other) by gavage in corn oil on 10 consecutive days. A corn oil control group was also included. At sacrifice, liver and kidney samples were collected and analyzed for cyanide-insensitive palmitoyl CoA oxidase activity as a marker for peroxisome proliferation. The individual chemicals produced statistically significantly increased palmitoyl CoA oxidase activity in rat and mouse liver and kidney compared with controls (except for tetrachloroethylene alone in rat kidney). In animals exposed to trichloroethylene alone, palmitoyl CoA oxidase activity values (expressed as a percentage of control values) were 239 and 261% for rat liver and kidney, respectively, and 625 and 360% for mouse liver and kidney, respectively. In animals exposed to tetrachloroethylene alone, values were 167 and 87% for rat liver and kidney, respectively, and 428 and 232% for mouse liver and kidney, respectively. In animals exposed to the mixture, values were 263 and 319% for rat liver and kidney, respectively, and 460 and 232% for mouse liver and kidney, respectively. In each case except rat kidney values, the response of the peroxisome proliferation marker to the mixture was less than the sum of the responses to the individual chemicals. Goldsworthy and Popp (1987) concluded that joint administration of these two chemicals did not produce additive or synergistic effects and did not differ from administration of the chemicals individually; however the results seem to be more consistent with a less-than-additive joint action of tetrachloroethylene and trichloroethylene on peroxisomal proliferation at the dose levels tested. Trichloroacetic acid, a metabolite of both chemicals via initial catalysis by CYP isozymes, is expected to be the responsible agent for producing the observed increase in peroxisomal enzyme activity. It is plausible that tetrachloroethylene may competitively or non-competitively inhibit the binding of trichloroethylene to the active sites of CYP isozymes, and slow the overall rate of formation of trichloroacetic acid.

Jonker et al. (1996) tested the hypothesis of dose addition with regard to renal effects produced by gavage of rats for 32 days with ternary or quaternary mixtures including trichloroethylene, tetrachloroethylene, and two other chemicals also thought to produce renal effects by a mechanism involving conjugation with glutathione and β-lyase hydrolysis (hexachloro-1,3-butadiene and 1,1,2-trichloro3,3,3-trifluoropropene). Relative kidney weight in rats was increased to a similar extent by the four chemicals at their renal

LOAEL values, by a mixture of the four chemicals together at 1/4 of their renal LOAEL values (one toxicity unit: 600 mg/kg/day tetrachloroethylene, 500 mg/kg/day trichloroethylene) and by ternary mixtures of the chemicals at 1/3 of their renal LOAEL values (one toxicity unit: 800 mg/kg/day tetrachloroethylene, 667 mg/kg/day trichloroethylene). This result is consistent with the renal toxicity of the mixture being determined by dose additivity. Other endpoints indicative of renal toxicity (histopathology and numerous urinanalytic variables such as glucose, total protein, and alkaline phosphatase) were not affected by exposure to the ternary (at 1/3 LOAEL values) or quaternary (at 1/4 LOAEL values) mixtures. Because the individual chemicals differentially affected these endpoints at the renal LOAEL values (e.g., tetrachloroethylene and trichloroethylene produced renal multifocal vacuolation, but the other two chemicals did not), the results were unsuitable for assessing the mode of joint action on these endpoints. Rats exposed to quaternary mixtures with components at ½ LOAEL values (two toxicity units) displayed clear renal toxicity in many endpoints including increased renal weight, decreasing urinary concentration ability, increased urinary excretion of protein, glucose, and various enzymes, and renal multifocal vacuolation. However, the lack of data for exposures to single chemical doses at two toxicity units in this study precluded further assessment of the additivity hypothesis. In general, this study provides evidence that trichloroethylene and tetrachloroethylene, along with two other similarly acting nephrotoxicants, jointly act in an additive manner in affecting kidney weight.

In summary, a study of urinary metabolites in workers exposed to tetrachloroethylene alone, trichloroethylene alone, or a mixture of tetrachloroethylene and trichloroethylene provides *in vivo* evidence that tetrachloroethylene inhibits the metabolism of trichloroethylene in humans (Seiji et al. 1989). This observation is consistent with results of a rat study indicating that tetrachloroethylene and trichloroethylene jointly act in a less-than-additive manner in inducing hepatic and renal peroxisomal proliferation, a response to trichloroacetic acid, a major metabolite of both chemicals (Goldsworthy and Popp 1987). The results in rats may be partly explained by tetrachloroethylene competitively or non-competitively inhibiting the binding of trichloroethylene to active sites of CYP enzymes and slowing the overall rate of appearance of trichloroacetic acid (compared with the rate with trichloroethylene exposure alone) due to its slower rate of conversion to trichloroacetic acid. The less-than-additive interaction in rats was observed at high exposure levels relative to environmental levels. If the observed interaction is competitive, it is unlikely to occur at lower exposure levels, when substrate concentrations are not in excess of hepatic CYP enzyme levels. In PBPK model simulations of competitive metabolic interactions between trichloroethylene and vinyl chloride, less-than-additive interaction occurred only at concentrations of substrates in excess of hepatic enzyme levels (Barton et al. 1995). In contrast, evidence of tetrachloroethylene inhibition of trichloroethylene metabolism has been reported in workers exposed to

both chemicals at low (<20 ppm) exposure levels (Seiji et al. 1989). A possible explanation of this finding is that tetrachloroethylene may inhibit trichloroethylene metabolism by a non-competitive mechanism that operates at low and high exposure levels.

Other rat studies of possible interactions between tetrachloroethylene and trichloroethylene in affecting liver or kidney endpoints include one indicating that the chemicals, along with two other chemicals that are also thought to produce renal effects by a mechanism involving conjugation with glutathione and β-lyase hydrolysis, additively act to increase kidney weight (Jonker et al. 1996), and another, of inadequate design to determine joint toxic action, that showed that a mixture of subthreshold doses of tetrachloroethylene and trichloroethylene could produce adverse effects on liver and kidney endpoints (Stacey 1989). Overall, the available weight-of-evidence suggests that coexposure of humans to tetrachloroethylene and trichloroethylene may inhibit the metabolism of trichloroethylene and thereby may inhibit carcinogenic and noncarcinogenic responses in the liver and kidney to trichloroethylene metabolites. However, the significance of this metabolic interaction to the nervous system effects (central nervous system depression) from trichloroethylene is not obvious since these effects have been attributed to both the parent chemical and a metabolite of trichloroethylene, (i.e., trichloroethanol). It is plausible that both parent chemicals and trichloroethanol may jointly act in an additive manner to produce nervous system effects, but studies designed to test this hypothesis were not located. The available data provide no direct evidence that trichloroethylene influences the metabolism of tetrachloroethylene (or its liver or kidney toxicity), but the limited capacity for tetrachloroethylene metabolism suggests that any influence of trichloroethylene on tetrachloroethylene metabolism should be of little toxicological significance.

Table 3. Summary of Available Data on the Influence of Trichloroethylene on Toxicity/Carcinogenicity of Tetrachloroethylene and the Influence of Tetrachloroethylene on Toxicity/Carcinogenicity of Trichloroethylene after Simultaneous Exposure

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Trichloroethylene Influence on Toxicity/Carcinogenicity of Tetrachloroethylene						
Inhalation Exposure (ppm)[a]						
Repeated Exposure	urinary excretion of metabolites		9.4 + 16.6 (h)[b]		No indication of trichloroethylene influence on tetrachloroethylene metabolism	Seiji et al. 1989
Oral Exposure (mg/kg/day)[a]						
Repeated Exposure	liver and kidney palmitoyl CoA oxidase activity			1,000 + 1,000 (r)[b] 1,000 + 1,000 (m)[b]	Less-than-additive joint action. May be due to tetrachloroethylene inhibition of trichloroethylene metabolism via CYP isozymes	Goldsworthy and Popp 1987
Repeated Exposure	kidney-to-body weight ratio		500 + 600 (r)[b]		Additive joint action with two other chemicals	Jonker et al. 1996
Intraperitoneal Exposure (mg/kg/day)[a]						
acute	serum ALT and SDH, liver-to-BW ratio		1,314 + 2,487 (r)[b]		Joint action is indeterminate due to study design limitation	Stacey 1989

Table 3. Summary of Available Data on the Influence of Trichloroethylene on Toxicity/Carcinogenicity of Tetrachloroethylene and the Influence of Tetrachloroethylene on Toxicity/Carcinogenicity of Trichloroethylene after Simultaneous Exposure *(continued)*

Duration	Endpoint	Results			Conclusions	References
		Greater than additive	Additive/no effect	Less than additive		
Tetrachloroethylene Influence on Toxicity/Carcinogenicity of Trichloroethylene						
Inhalation Exposure (ppm)[a]						
Repeated Exposure	urinary excretion of metabolites			16.6 + 9.4 (h)[b]	Less than additive metabolic interaction	Seiji et al. 1989
Oral Exposure (mg/kg/day)[a]						
Repeated Exposure	liver and kidney palmitoyl CoA oxidase activity			1,000 + 1,000 (r)[b] 1,000 + 1,000 (m)[b]	Less-than-additive joint action. May be due to tetrachloroethylene inhibition of trichloro-ethylene metabolism via CYP isozymes	Goldsworthy and Popp 1987
Repeated Exposure	kidney-to-body weight ratio		600 + 500 (r)[b]		Additive joint action with two other chemicals	Jonker et al. 1996
Intraperitoneal Exposure (mg/kg/day)[a]						
acute	serum ALT and SDH, liver-to-BW ratio		2,487 + 1,314 (r)[b]		Joint action is indeterminate due to study design limitation.	Stacey 1989

[a] First dose listed is for the chemical influencing the other chemical's toxicity/carcinogenicity.
[b] Species code: h = human; m = mouse; r = rat

ALT = alanine aminotransferase; BW = body weight; SDH = sorbitol dehydrogenase

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

1,1,1-Trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene are frequently encountered together at hazardous waste sites. Acute or repeated inhalation exposure to any of these chemicals starting at concentrations as low as 20–100 ppm is expected to produce neurological impairment resulting from the parent chemicals (and a metabolite in the case of trichloroethylene) acting on components of neuronal membranes (see Appendices A, B, C, and D). Animal studies also provide evidence, of varying weights for the four chemicals, that repeated inhalation exposure at high exposure levels (>100–500 ppm) can damage liver and kidney tissue and produce cancer due to the formation of reactive metabolites (see Appendices A, B, C, and D). Table 4 shows that neurological impairment forms the basis for ATSDR's inhalation MRLs for these chemicals. Acute oral exposure to trichloroethylene or tetrachloroethylene during pregnancy is also thought to present a hazard to the neurological development of offspring, and these effects form the basis of the oral MRLs for these chemicals (see Table 4). It should be noted, however, that significant data gaps exist with regard to deriving MRLs for the four chemicals. For example, no MRLs of any kind have been derived for 1,1-dichloroethane due to inadequate data.

There is only limited weight of evidence that inhalation or oral exposures to these chemicals may present significant cancer risks to humans. Reflecting this limited weight, EPA (IRIS 2001) assigned cancer classifications of Group D (Not Classifiable as to Human Carcinogenicity) to 1,1,1-trichloroethane, Group C (Possible Human Carcinogen) to 1,1-dichloroethane, and an intermediate B2/C (Probable/Possible Human Carcinogen) to trichloroethylene and tetrachloroethylene, and lists no oral slope factor or inhalation unit risk for these chemicals on the IRIS (2001) database. Part of the uncertainty concerning the possible carcinogenicity of these chemicals in humans arises from evidence that reactive metabolites are responsible for observed carcinogenic responses in rodents and that responsive species more readily metabolize these chemicals than humans.

In the absence of pertinent data on neurological responses to mixtures of all four chemicals and PBPK models that predict the direction and magnitude of interactions among the four chemicals, health hazards from inhalation or oral exposure to mixtures of these chemicals may best be assessed by a components-based approach such as the Hazard Index approach (ATSDR 2001a). Such an approach requires judgements concerning the presence or absence of interactions affecting the response of the shared apparent critical target organ, the nervous system, and other shared targets, the liver and kidney.

Table 4. Health Effects Forming the Basis of ATSDR Inhalation and Oral MRLs for Chemicals of Concern
(Source: ATSDR 1990, 1995, 1997a, 1997b)

Chemical	Inhalation MRLs			Oral MRLs		
	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
1,1,1-Trichloro-ethane	neuro-behavioral changes in humans	brain chemistry changes in gerbils	ND	ND	ND	ND
1,1-Dichloro-ethane	ND	ND	ND	ND	ND	ND
Trichloro-ethylene	neuro-behavioral changes in humans	neuro-behavioral changes in rats	ND	neuro-behavioral changes in rat offspring	ND	ND
Tetra-chloroethylene	neuro-behavioral changes in humans	ND	neuro-behavioral changes in humans	neuro-behavioral changes in mouse offspring	ND	ND

ND = None derived due to inadequate data

No studies were located that examined neurological endpoints and described dose-response relationships in humans or animals following exposure to mixtures of all four of these chemicals, but mechanistic data and interactions data have been evaluated in Section 2.2 to determine how pairs of these chemicals may jointly act in producing nervous system, liver, and kidney effects. To characterize the overall potential for interactions among 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloro-ethylene, four binary weight-of-evidence determinations (BINWOEs) were derived for each of the six pairs of chemicals (two for the effects of chemical A on the nervous system effects of chemical B and the liver and kidney toxicity of B, and the other two for the effects of B on the toxicities of A) using the classification scheme described by ATSDR (2001b) and shown in Figure 1. The BINWOEs are described in the text of Tables 5 to 16. Tables 17 and 18 summarize the BINWOE determinations for noncancer effects on the nervous system (the apparent shared critical toxicity target of all four chemicals) and noncancer and cancer effects on the liver and kidney (shared cancer targets of several of the chemicals in animals), respectively.

No studies were located that directly examined joint action of the chemicals on the nervous system, but mechanistic understanding indicates that each of the chemicals is expected to produce nervous system effects by reversible actions of parent chemicals (and the trichloroethylene metabolite, trichloroethanol) on neuronal membrane components. Nervous system depression from lipophilic solvents such as the chemicals of concern for this document is thought to involve reversible intercalation in lipid bilayers of nerve membranes (yielding changes in membrane fluidity) and/or reversible interactions with membrane proteins (yielding conformational changes) leading to altered ion transport, enzymic activities, and neurotransmitter receptor functions necessary for normal nerve impulses and regeneration of action potentials (Balster 1998; Cruz et al. 1998; Engelke et al. 1996; Franks and Lieb 1985, 1987; Mihic et al. 1994; von Euler 1994). Based on the plausibility that the chemicals may act jointly in an additive manner via the same mechanisms of action in affecting neuronal membranes, each of the BINWOEs for nervous system effects determined an additive joint action with data quality factors of "II" for mechanistic understanding to reflect moderate mechanistic understanding and "C" for toxicologic significance to reflect lack of studies designed to test the hypothesis of joint additive actions on the nervous system (see Table 17).

BINWOE determinations were made for noncancer and cancer effects in the liver and kidney and are discussed in Tables 5 to 16. Based on results from animal studies, each of the chemicals is expected to produce noncancer and cancer effects in the liver and/or kidney via reactive metabolites formed under high exposure chronic conditions. BINWOE determinations for liver and kidney endpoints were made in anticipation of public health concerns that there might be greater-than-additive interactions that might cause liver and kidney effects to occur. The analysis of the available data, however, provides no indication that this type of interaction might occur. Additive joint action was determined in 11 of the 12 BINWOEs (see Table 18) based on plausibility from mechanistic understanding and/or limited evidence from rat studies examining joint action on liver or kidney endpoints (e.g., Stacey 1989). The twelfth BINWOE, for the effect of tetrachloroethylene on trichloroethylene (Table 16), was determined as a less-than-additive joint action (i.e., tetrachloroethylene may antagonize trichloroethylene-induced liver and kidney effects by inhibiting the formation of trichloroacetic acid from trichloroethylene) based on *in vivo* evidence that tetrachloroethylene inhibits the metabolism of trichloroethylene in humans under occupational exposure conditions (Seiji et al. 1989), and tetrachloroethylene and trichloroethylene acted in a less-than-additive manner in causing hepatic and renal peroxisomal proliferation in orally exposed rats (Goldsworthy and Popp 1987).

The use of a Target Toxicity Dose approach does not appear to be warranted because neurological effects are the basis for all of ATSDR's MRLs for these chemicals regardless of exposure duration or route, and there is no evidence for greater-than-additive interactions in the liver and kidney, which also are shared targets of the chemicals.

Figure 1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Classification		Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	–1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
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.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur is 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.	0.71
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.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from –1 through 0 to +1

* Source: ATSDR 2001a

Table 5. Effect of **1,1,1-Trichloroethane** on **1,1-Dichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that 1,1,1-trichloroethane and 1,1-dichloroethane may jointly act in an additive manner to depress the central nervous system and sensitize the heart to epinephrine, but studies designed to test this hypothesis were not located. Although reactive metabolites of both chemicals are expected to produce liver and kidney effects, neither chemical is extensively metabolized, nor hepatotoxic except at high exposure levels. Additive joint action to produce effects on the liver or kidney is plausible.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices A and B; ATSDR 1990, 1995). They may act jointly in an additive manner to produce central nervous system depression and heart sensitization to epinephrine, but studies designed to test this hypothesis were not located.

Mild liver or kidney effects observed in rodents from either of these chemicals are believed to be caused by reactive metabolic intermediates formed via CYP 2B1/2 or 2E1 catalysis (see Appendices A and B). These chemicals are of low potency because they are poorly metabolized (Kaneko et al. 1994; McCall et al. 1983; Mitoma et al. 1985; Nolan et al. 1984). Any influence (e.g., inhibition at CYP active sites or induction of Phase I or Phase II enzymes) that they may have on each other's metabolism should have little influence on their toxicity, since they are poorly metabolized, downstream enzymes may prevent elevation of intracellular concentrations of putative toxic metabolites, and repair mechanisms may efficiently fix any damage to cellular macromolecules. Studies directly designed to examine how they may influence each other's metabolism or toxicity, however, were not located.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - No interaction data for 1,1,1-trichloroethane and 1,1-dichloroethane were located for any toxicological endpoints; thus, the lowest possible toxicologic significance data quality factor, C, was assigned to both BINWOE determinations.

Additional Uncertainties - Competitive metabolic interactions may exist between these chemicals at CYP isozyme catalytic sites under high exposure conditions when CYP catalytic sites are saturated. Pretreatment of rats with phenobarbital or ethanol increased rates of 1,1-dichloroethane metabolism in liver microsomes (McCall et al. 1983; Sato et al. 1980), but the possible influence of CYP induction on 1,1-dichloroethane is unexamined. Furthermore, 1,1,1-trichloroethane did not induce hepatic CYP2E1 or 2B1/2 in rats until doses approached lethal levels above 500 mg/kg (Bruckner et al. 2000). The limited degree to which 1,1-dichloroethane is metabolized suggests that any interactions should have little influence on toxicity.

Table 6. Effect of **1,1-Dichloroethane** on **1,1,1-Trichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that 1,1,1-trichloroethane and 1,1-dichloroethane may jointly act in an additive manner to depress the central nervous system and sensitize the heart to epinephrine, but studies designed to test this hypothesis were not located. Although reactive metabolites of both chemicals are expected to produce liver and kidney effects, neither chemical is extensively metabolized, nor hepatotoxic except at high exposure levels. Additive joint action to produce effects on the liver or kidney is plausible.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices A and B; ATSDR 1990, 1995). They may act jointly in an additive manner to produce central nervous system depression and heart sensitization to epinephrine, but studies designed to test this hypothesis were not located.

Mild liver or kidney effects observed in rodents from either of these chemicals are believed to be caused by reactive metabolic intermediates formed via CYP 2B1/2 or 2E1 catalysis (see Appendices A and B). These chemicals are of low potency because they are poorly metabolized (Kaneko et al. 1994; McCall et al. 1983; Mitoma et al. 1985; Nolan et al. 1984). Any influence (e.g., inhibition at CYP active sites or induction of Phase I or Phase II enzymes) that they may have on each other's metabolism should have little influence on their toxicity, since they are poorly metabolized, downstream enzymes may prevent elevation of intracellular concentrations of putative toxic metabolites, and repair mechanisms may efficiently fix any damage to cellular macromolecules. Studies directly designed to examine how they may influence each other's metabolism or toxicity, however, were not located.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - No interaction data for 1,1-dichloroethane and 1,1,1-trichloroethane were located; thus, the lowest possible toxicologic significance data quality factor, C, was assigned to both BINWOE determinations.

Additional Uncertainties - Competitive metabolic interactions may exist between these chemicals at CYP isozyme catalytic sites under high exposure conditions when CYP catalytic sites are saturated. Pretreatment of rats with phenobarbital or ethanol to increase rates of 1,1,1-trichloroethane metabolism in liver microsomes has not shown a consistent potentiation of hepatotoxicity (Carlson 1973; Cornish et al 1973). Rates of 1,1,1-trichloroethane metabolism in rats, even under induced conditions (ethanol pretreatment), are much lower than rates for highly metabolized chlorinated hydrocarbons such as trichloroethylene (Kaneko et al. 1994). The limited degree to which 1,1,1-trichloroethane is metabolized suggests that any interactions should have little influence on toxicity.

Table 7. Effect of **1,1,1-Trichloroethane** on **Trichloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIB
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - There are limited data to suggest that these chemicals may jointly act in an additive manner to produce effects on the central nervous system, the liver, and the kidney.

Mechanistic Understanding - Like other solvents, the parent chemicals (and a trichloroethylene metabolite, trichloroethanol) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices A and C, ATSDR 1995, 1997a). They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Noncancer or cancer effects in the liver and kidney from trichloroethylene or 1,1,1-trichloroethane are thought to be caused by reactive metabolites, produced via CYP catalysis (see Appendices A and C). CYP2E1 is the predominant isozyme involved in Phase I metabolism of both chemicals, but trichloroethylene is metabolized to a much greater extent than 1,1,1-trichloroethane (Kaneko et al. 1994; Lash et al. 2000). Although phenobarbital induction of CYP has been associated with enhancement of acute high level trichloroethylene hepatotoxicity (Allemand et al. 1978; Carlson 1974; Moslen et al. 1977; Nakajima et al. 1990), 1,1,1-trichloroethane is not expected to influence Phase I trichloroethylene metabolism or toxicity, especially at environmentally relevant exposure levels (see Additional Uncertainties section below). In rats exposed by inhalation to a mixture of 500 ppm 1,1,1-trichloroethane and 200 ppm trichloroethylene, trichloroethylene metabolism did not appear to be impaired (Vainio et al. 1978). This study, however, did not include a trichloroethylene-alone exposure group. Limited data from rat studies indicate that they may jointly act on the liver and kidney (see Section 2.2.3; Stacey 1989). Mechanistic understanding was assigned a moderate data quality factor of II to reflect the lack of data regarding joint action on the nervous system and data availability regarding joint action on the liver and kidney.

Toxicological Significance - There is limited evidence from rat *in vitro* and *in vivo* studies (Stacey 1989) that reactive metabolites of these chemicals may jointly act in an additive manner to produce liver and kidney damage, but the study design could not definitively rule out greater-than-additive or less-than-additive interactions (see Section 2.2.3). To reflect the availability of these data (even though they are ambiguous), a medium data quality factor, B, was assigned. To reflect the lack of data on joint action on the nervous system, the lowest possible data quality factor, C, was assigned.

Additional Uncertainties - The effects of trichloroethylene on the central nervous system may involve not only the parent chemical, but also metabolites such as trichloroethanol. Competitive metabolic interaction between these chemicals are possible under conditions saturating CYP catalytic sites, but 1,1,1-trichloroethane is likely to be a poor competitor of trichloroethylene given that it is much more slowly metabolized than trichloroethylene. Furthermore, alteration of trichloroethylene metabolism at CYP catalytic sites is likely to be physiologically important only at high exposure levels when substrate concentrations are in excess of catalytic sites. For example, CYP induction by ethanol only altered trichloroethylene metabolic rates in rats exposed to high (500–1,000 ppm), but not low (50–100 ppm), trichloroethylene levels (Kaneko et al. 1994). 1,1,1-Trichloroethane modestly and transiently induced hepatic CYP 2B1/2 and CYP2E1 in rats only at lethal dose levels (5 or 10 g/kg; Bruckner et al. 2000).

Table 8. Effect of **Trichloroethylene** on **1,1,1-Trichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIB
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - There are limited data to suggest that these chemicals may jointly act in an additive manner to produce effects on the central nervous system, the liver, and the kidney.

Mechanistic Understanding - Like other solvents, the parent chemicals (and a trichloroethylene metabolite, trichloroethanol) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias. They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Noncancer or cancer effects in the liver or kidney from trichloroethylene or 1,1,1-trichloroethane are thought to be caused by reactive metabolites via CYP catalysis (see Appendices A and C). CYP2E1 is the predominant isozyme involved in Phase I metabolism of both chemicals, but trichloroethylene is metabolized to a much greater extent than 1,1,1-trichloroethane (Kaneko et al. 1994; Lash et al. 2000). 1,1,1-Trichloroethane is not a potent toxicant to the liver or kidney because it is poorly metabolized. Trichloroethylene enhancement of 1,1,1-trichloroethane metabolism or hepatotoxicity is not expected. Only high doses of trichloroethylene (>400 mg/kg), not low doses (8 mg/kg), induced hepatic CYP2E1 in rats (Lee et al. 2000), and phenobarbital or ethanol induction to enhance hepatic 1,1,1-trichloroethane metabolism in rats has not produced consistent evidence of potentiation of 1,1,1-trichloroethane hepatotoxicity (Carlson et al. 1973; Cornish et al. 1973). Limited data from rat studies indicate that the two chemicals may jointly act on the liver and kidney (Stacey 1989; see Section 2.2.3). Mechanistic understanding was assigned a data quality factor of II to reflect the lack of data regarding joint action on the nervous system and data availability regarding joint action on the liver and kidney.

Toxicological Significance - There is limited evidence from rat *in vitro* and *in vivo* studies (Stacey 1989) that reactive metabolites of these chemicals may jointly act in an additive manner to produce liver and kidney damage, but the study design could not definitively rule out greater-than-additive or less-than-additive interactions (see Section 2.2.3). To reflect the availability of these data (even though they are ambiguous), a medium data quality factor, B, was assigned. To reflect the lack of data on joint action on the nervous system, the lowest possible data quality factor, C, was assigned.

Additional Uncertainties - In general, 1,1,1-trichloroethane is poorly metabolized, but metabolism can be influenced by induction of CYP isozymes. For example, CYP induction by ethanol increased metabolism of inhaled 1,1,1-trichloroethane in rats, but most of the chemical was still eliminated unmetabolized (Kaneko et al. 1994). Thus, any possible interference or enhancement that trichloroethylene may have on 1,1,1-trichloroethane metabolism should have little influence on 1,1,1-trichloroethane toxicity. Furthermore, the balance of Phase I and II metabolism should determine whether or not toxicity is expressed. Capabilities of downstream enzymes may be sufficient to prevent increased concentrations of putative toxic intermediate metabolites of 1,1,1-trichloroethane, even under conditions of Phase I induction.

Table 9. Effect of **Tetrachloroethylene** on **1,1,1-Trichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIB
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - There are limited data to suggest that these chemicals may jointly act in an additive manner to produce effects on the central nervous system, liver, and kidney.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices A and D; ATSDR 1995, 1997b). They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Liver and kidney effects from these chemicals are expected to involve reactive intermediates formed via CYP catalysis (CYP 2E1 and 2B1/2) in the liver or hydrolysis by β-lyase of a glutathione conjugate in the kidney, but neither chemical is a potent toxicant in these tissues because they are poorly metabolized (see Appendices A and D). Tetrachloroethylene inhibited the rates of urinary excretion of a 1,1,1-trichloroethane metabolite, trichloroethanol, in rats exposed by inhalation to a mixture of 350 ppm 1,1,1-trichloroethane and 100 ppm tetrachloroethylene (Koizumi et al. 1982). The limited degree to which 1,1,1-trichloroethane is metabolized indicates that any influence that tetrachloroethylene may have on 1,1,1-trichloroethane metabolism should have little influence on toxicity, because downstream enzymes may prevent elevation of intracellular concentrations of putative toxicants and repair mechanisms may fix any damage to cellular macromolecules. Limited data from rat studies indicate that they may jointly act on the liver and kidney (see Section 2.2.4; Stacey 1989). Mechanistic understanding was assigned a data quality factor of II to reflect the lack of data regarding joint action on the nervous system and data availability regarding joint action on the liver and kidney (see below).

Toxicological Significance - Due to the lack of data on joint action on the nervous system, the lowest possible data quality factor, C, was assigned. Limited evidence from rat *in vitro* and *in vivo* studies (Stacey 1989) suggests that reactive metabolites of these chemicals may jointly act in an additive manner to produce liver damage, but the study design could not definitively rule out greater-than-additive or less-than-additive interactions (see Section 2.2.4.) To reflect the availability of these data (even though they are ambiguous), a moderate data quality factor, B, was assigned.

Additional Uncertainties - No information was located to indicate whether tetrachloroethylene may enhance the metabolism of 1,1,1-trichloroethane. CYP induction by ethanol has not produced consistent potentiation of acute high-level 1,1,1-trichloroethane hepatotoxicity (Carlson 1973; Cornish et al. 1973). Competitive interactions at CYP isozymes are possible, especially at high concentrations saturating catalytic sites. However, any interference or enhancement of 1,1,1-trichloroethane metabolism should have little influence on toxicity in the liver or kidney.

Table 10. Effect of **1,1,1-Trichloroethane** on **Tetrachloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIB
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - There are limited data to suggest that these chemicals may jointly act in an additive manner to produce effects on the central nervous system, liver, and kidney.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices A and D; ATSDR 1995, 1997b). They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Liver and kidney effects from these chemicals are believed to involve reactive intermediates formed via CYP catalysis (CYP 2E1 and/or 2B1/2) in the liver or hydrolysis by β-lyase of a glutathione conjugate in the kidney, but neither chemical is a potent toxicant in these tissues because they are poorly metabolized (see Appendices A and D; ATSDR 1995; 1997b). The limited degree to which either of these chemicals is metabolized indicates that any influence that they may have on each other's metabolism should have little influence on their toxicity, because downstream enzymes may prevent elevation of intracellular concentrations of putative toxicants and repair mechanisms may fix any damage to cellular macromolecules. Limited data from rat studies indicate that they may jointly act on the liver and kidney (see Section 2.2.4; Stacey 1989). Mechanistic understanding was assigned a data quality factor of II to reflect the lack of data regarding joint action on the nervous system and data availability regarding joint action on the liver and kidney (see below).

Toxicological Significance - Due to the lack of data on joint action on the nervous system, the lowest possible data quality factor, C, was assigned. Limited evidence from rat *in vitro* and *in vivo* studies (Stacey 1989) suggests that reactive metabolites of these chemicals may jointly act in an additive manner to produce liver damage, but the study design could not definitively rule out greater-than-additive or less-than-additive interactions (see Section 2.2.4.) To reflect the availability of these data (even though they are ambiguous), a moderate data quality factor, B, was assigned.

Additional Uncertainties - 1,1,1-Trichloroethane modestly and transiently induced hepatic CYP2E1 and 2B1/2 levels in rats at exposure levels >500 mg/kg (Bruckner et al. 2000). CYP induction by ethanol, phenobarbital, or PCBs has not produced consistent potentiation of acute high-level tetrachloroethylene hepatotoxicity (Cornish and Adefuin 1966; Cornish et al. 1973; Klaassen and Plaa 1966; Moslen et al. 1977). Competitive interactions between 1,1,1-trichloroethane and tetrachloroethylene at CYP isozymes are possible, especially at high concentrations saturating catalytic sites. However, any possible interference or enhancement that 1,1,1-trichloroethane may have on tetrachloroethylene metabolism should have little influence on toxicity, because tetrachloroethylene is poorly metabolized.

Table 11. Effect of **1,1-Dichloroethane** on **Trichloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that 1,1-dichloroethane and trichloroethylene may jointly act in an additive manner to produce central nervous system effects, liver effects, and kidney effects, but studies designed to test this hypothesis were not located.

Mechanistic Understanding - Like other solvents, the parent chemicals (and tetrachloroethanol, a metabolite of trichloroethylene) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices B and C; ATSDR 1990, 1997a). They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Development of liver and kidney damage and cancer effects in animals from trichloroethylene are believed to involve reactive intermediates produced via CYP catalysis in the liver or β-lyase hydrolysis of a glutathione conjugate (of trichloroethylene) in the kidney (see Appendix C). Phenobarbital induction of CYP has been associated with enhancement of acute high level trichloroethylene hepatotoxicity (Allemand et al. 1978; Carlson 1974; Moslen et al. 1977), but 1,1-dichloroethane is not expected to influence Phase I trichloroethylene metabolism or hepatotoxicity (see Additional Uncertainties section below). Studies designed to examine if coexposure to 1,1-dichloroethane would influence trichloroethylene metabolism or toxicity were not located, but joint additive action on the liver or kidney is plausible.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - Due to the lack of data on joint action on the nervous system, liver, and kidney, the lowest possible data quality factor, C, was assigned for each BINWOE.

Additional Uncertainties - Competitive metabolic interactions between 1,1-dichloroethane and trichloroethylene at CYP-catalyzed reactions are possible under high exposure conditions saturating CYP catalytic sites. However, 1,1-dichloroethane should be a poor competitor of trichloroethylene because it is much more slowly metabolized than trichloroethylene (see Appendices A and C). No data are available to indicate whether 1,1-dichloroethane may enhance trichloroethylene metabolism, but demonstration of metabolic enhancement alone is an insufficient condition for predicting greater-than-additive interaction due to possibilities of detoxification by downstream enzymes and/or repair of damaged cellular macromolecules. Furthermore, any possible alteration (inhibition or enhancement) of trichloroethylene metabolism by 1,1-dichloroethane is likely to be physiologically important only at high exposure levels when trichloroethylene concentrations are in excess of CYP catalytic sites (Kaneko et al. 1994).

Table 12. Effect of **Trichloroethylene** on **1,1-Dichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that trichloroethylene and 1,1-dichloroethane may jointly act in an additive manner to produce central nervous system effects, liver effects, and kidney effects, but studies designed to test this hypothesis were not located.

Mechanistic Understanding - Like other solvents, the parent chemicals (and trichloroethanol, a metabolite of trichloroethylene) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices B and C; ATSDR 1990, 1997a) The chemicals may act jointly in an additive manner to cause these effects, but studies designed to test this hypothesis were not located.

Development of liver or kidney effects in rodents from these chemicals are believed to involve reactive metabolic intermediates formed via CYP catalysis in the liver or β-lyase hydrolysis of a glutathione conjugate (of trichloroethylene) in the kidney (see Appendices B and C). 1,1-Dichloroethane is of low potency because it is poorly metabolized *in vivo* (Mitoma et al., 1985) and may not be conjugated to glutathione to the same degree as its more toxic isomer, 1,2-dichloroethane (McCall et al., 1983). Any influence (e.g., inhibition at CYP active sites or induction of Phase I or Phase II enzymes) that trichloroethylene may have on 1,1-dichloroethane metabolism should have little influence on its toxicity, due to this low rate of metabolism, downstream metabolism that may prevent elevation of concentrations of potentially toxic intermediates, and repair mechanisms that may fix damaged cellular macromolecules. Studies directly designed to examine how trichloroethylene and 1,1-dichloroethane may influence each other's metabolism or toxicity, however, were not located.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - Due to the lack of data on joint action on the nervous system, liver, and kidney, the lowest possible data quality factor, C, was assigned for each BINWOE.

Additional Uncertainties - Due to overlap in metabolic reactions catalyzed by CYP isozymes, competitive metabolic interactions between 1,1-dichloroethane and trichloroethylene are possible under high exposure conditions saturating CYP catalytic sites. Pretreatment of rats with phenobarbital increased rates of CYP-mediated metabolism of 1,1-dichloroethane in liver microsomes (McCall et al. 1983), but the influence of CYP induction on 1,1-dichloroethane toxicity is unexamined. 1,1,1-Trichloroethylene induced hepatic levels of CYP2E1 in rats only at high doses (>400 mg/kg) and not at low doses (8 mg/kg) (Lee et al., 2000). The limited degree to which 1,1-dichloroethane is metabolized suggests that any influence that trichloroethylene may exert on 1,1-dichloroethane metabolism should have minimal influence on toxicity.

Table 13. Effect of **1,1-Dichloroethane** on **Tetrachloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that tetrachloroethylene and 1,1-dichloroethane may jointly act in an additive manner to produce central nervous system effects, liver effects, and kidney effects, but studies designed to test this hypothesis were not located.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices B and D; ATSDR 1990, 1997b) . They may act jointly in an additive manner to produce these effects, but studies designed to test this hypothesis were not located.

Liver or kidney effects observed in rodents repeatedly exposed to high levels of either of these chemicals are believed to be caused by reactive metabolic intermediates formed via CYP 2B1/2 or 2E1 catalysis or β-lyase hydrolysis of a glutathione conjugate (see Appendices B and D). These chemicals are of low potency because they are poorly metabolized (Mitoma et al., 1985; Monster et al. 1979; Pegg et al. 1979). Any influence (e.g., inhibition at CYP active sites or induction of Phase I or Phase II enzymes) that they may have on each other's metabolism should have little influence on their toxicity, because they are poorly metabolized, downstream enzymes may prevent elevation of intracellular concentrations of metabolic toxicants, and repair mechanisms may efficiently fix any damage to cellular macromolecules. Studies directly designed to examine how they may influence each other's metabolism or toxicity, however, were not located.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - Due to the lack of data on joint action on the nervous system, liver, and kidney, the lowest possible data quality factor, C, was assigned for each BINWOE.

Additional Uncertainties - Due to overlap in metabolic reactions catalyzed by CYP isozymes, competitive interactions between 1,1-dichloroethane and tetrachloroethylene are possible under high exposure conditions saturating CYP catalytic sites. CYP induction by ethanol, phenobarbital, or Aroclor 1254 has not produced consistent potentiation of acute high-level tetrachloroethylene hepatotoxicity (Cornish and Adefuin 1966; Cornish et al. 1973; Klaassen and Plaa 1966; Moslen et al. 1977). However, both chemicals are poorly metabolized, and any possible interference or enhancement that they may have on each other's metabolism should have little influence on their toxicity.

Table 14. Effect of **Tetrachloroethylene** on **1,1-Dichloroethane**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIC
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that tetrachloroethylene and 1,1-dichloroethane may jointly act in an additive manner to produce central nervous system effects, liver effects, and kidney effects, but studies designed to test this hypothesis were not located.

Mechanistic Understanding - Like other solvents, the parent chemicals depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias. The chemicals may act jointly in an additive manner to cause these effects, but studies designed to test this hypothesis were not located.

Liver or kidney effects observed in rodents repeatedly exposed to high levels of either of these chemicals are believed to be caused by reactive metabolic intermediates formed via CYP 2B1/2 or 2E1 catalysis or β-lyase hydrolysis of a glutathione conjugate (see Appendices B and D; ATSDR 1990, 1997b). These chemicals are of low potency because they are poorly metabolized (Mitoma et al., 1985; Monster et al. 1979; Pegg et al. 1979). Any influence (e.g., inhibition at CYP active sites or induction of Phase I or Phase II enzymes) that they may have on each other's metabolism should have little influence on their toxicity, because they are poorly metabolized, downstream enzymes may prevent elevation of intracellular concentrations of putative toxicants, and repair mechanisms may efficiently fix any damage to cellular macromolecules. Studies directly designed to examine how they may influence each other's metabolism or toxicity, however, were not located.

Mechanistic understanding was assigned a moderate data quality factor, II, to reflect lack of data regarding joint action on nervous system, liver, and kidney endpoints.

Toxicological Significance - Due to the lack of data on joint action on the nervous system, liver, and kidney, the lowest possible data quality factor, C, was assigned for each BINWOE.

Additional Uncertainties - Due to overlap in metabolic reactions catalyzed by CYP isozymes, competitive metabolic interactions between 1,1-dichloroethane and tetrachloroethylene are possible under high exposure conditions saturating CYP catalytic sites. Pretreatment of rats with phenobarbital or ethanol increased rates of 1,1-dichloroethane metabolism in liver microsomes (McCall et al. 1983; Sato et al. 1980), but the influence of CYP induction on acute or chronic 1,1-dichloroethane toxicity is unexamined. Both chemicals are poorly metabolized and any possible interference or enhancement that they may have on each other's metabolism should have little influence on their toxicity.

Table 15. Effect of **Trichloroethylene** on **Tetrachloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: =IIB
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that the parent chemicals and trichloroethanol (a metabolite of trichloroethylene) may additively act to produce nervous system effects, but studies designed to test this hypothesis were not located. It is plausible that trichloroethylene may have little influence on tetrachloroethylene metabolism, and that tetrachloroethylene and trichloroethylene metabolites would additively act to produce liver and kidney effects.

Mechanistic Understanding - Like other solvents, the parent chemicals (and the trichloroethylene metabolite, trichloroethanol) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices C and D; ATSDR 1997a, 1997b).

Liver or kidney effects in rodents exposed to high levels of tetrachloroethylene are believed to involve reactive metabolic intermediates (see Appendices C and D). Tetrachloroethylene is not a potent liver or kidney toxicant because it is poorly metabolized (Monster et al. 1979; Pegg et al. 1979). Any influence that trichloroethylene may have on tetrachloroethylene metabolism should have little influence on tetrachloroethylene toxicity due to detoxification from downstream metabolism and/or repair of damaged cellular macromolecules. Results from a rat and mouse study suggest that trichloroethylene and tetrachloroethylene act in a less-than-additive manner to cause hepatic and renal peroxisomal proliferation (Goldsworthy and Popp 1987; see Section 2.2.7). This observation may be explained by non-competitive inhibition of CYP isozymes leading to slower rates of trichloroacetic acid formation from trichloroethylene. Other rat studies (see Section 2.2.7) show that the chemicals act additively to increase kidney weight (Jonker et al. 1996), and mixtures of subthreshold doses can produce increased serum ALT (Stacey 1989). The latter observation could be consistent with additive joint action on the liver, but the study design could not definitively rule out greater-than-additive or less-than-additive joint action (Stacey 1989).

Mechanistic understanding was assigned a moderate quality factor (II) to reflect lack of data regarding joint actions on the nervous system, and uncertainties regarding joint actions on the liver and kidney.

Toxicological Significance - Studies designed to examine the joint toxic action of these chemicals on nervous system endpoints were not located. Thus, the lowest possible toxicologic significance data quality factor, C, was assigned for nervous system effects. For liver and kidney effects, a moderate data quality factor, B, was assigned because there are studies on the joint toxic action of these chemicals on liver and kidney endpoints in rats, but results are not consistent across endpoints (see above and Section 2.2.7).

Additional Uncertainties - Competitive metabolic interactions at CYP catalytic sites are possible, especially at high exposure levels when sites are saturated. CYP induction by ethanol, phenobarbital, or Aroclor 1254 has not produced consistent potentiation of acute high-level tetrachloroethylene hepatotoxicity (Cornish and Adefuin 1966; Cornish et al. 1973; Klaassen and Plaa 1966; Moslen et al. 1977). Any influence that trichloroethylene may have on tetrachloroethylene metabolism (enhancement or inhibition) should have little influence on toxicity, because tetrachloroethylene is poorly metabolized.

Table 16. Effect of **Tetrachloroethylene** on **Trichloroethylene**
BINWOE: =IIC
(for nervous system effects)
BINWOE: <IIB (-1 x 0.71 x 0.71= -0.50)
(for cancer and noncancer liver or kidney effects)

Direction of Interaction - It is plausible that the parent chemicals and trichloroethanol may jointly act in an additive manner to interact with nervous system membranes. There is evidence that tetrachloroethylene inhibits the metabolism of trichloroethylene in humans (Seiji et al. 1989) and evidence of less-than-additive joint action on hepatic and renal peroxisomal proliferation in rats and mice (Goldsworthy and Popp 1987). It is plausible that the interaction may antagonize liver and kidney effects from trichloroethylene metabolites.

Mechanistic Understanding - Like other solvents, the parent chemicals (and the trichloroethylene metabolite, trichloroethanol) depress nervous system functions by reversibly acting on neuronal membranes and sensitize the heart to epinephrine-induced arrhythmias (see Appendices C and D; ATSDR 1997a, 1997b). Mechanistic understanding was assigned a moderate quality factor (II) to reflect the lack of direct data on the joint action of these chemicals on the nervous system.

Liver or kidney effects in rodents exposed to high levels of these chemicals are believed to involve reactive metabolic intermediates (see Appendices C and D). Studies of urinary metabolites in workers exposed to trichloroethylene alone, tetrachloroethylene alone, or mixtures of trichloroethylene and tetrachloroethylene indicate that tetrachloroethylene inhibits the metabolism of trichloroethylene at low exposure levels (<20 ppm) (Seiji et al. 1989). Results from a rat and mouse study suggest that trichloroethylene and tetrachloroethylene act in a less-than-additive manner to cause hepatic and renal peroxisomal proliferation (Goldsworthy and Popp 1987). This observation may be explained by non-competitive inhibition of CYP isozymes leading to slower rates of trichloroacetic acid formation. Other rat studies show that the chemicals act additively to increase kidney weight (Jonker et al. 1996), and mixtures of subthreshold doses can produce increased serum ALT in rats (Stacey 1989; see Section 2.2.7). A moderate quality factor (II) was selected to reflect ambiguities (i.e., inconsistency of the database) regarding the projection of less-than-additive joint action on the liver and kidney.

Toxicological Significance - Studies designed to examine the joint toxic action of these chemicals on nervous system endpoints were not located. Thus, the lowest possible toxicologic significance data quality factor, C, was applied for nervous system effects. For liver and kidney effects, a moderate data quality factor, B, was selected. There is evidence for tetrachloroethylene inhibition of trichloroethylene metabolism in humans (Seiji et al. 1989), but evidence for less-than-additive joint action on liver and kidney endpoints in rats is not consistent across endpoints (see above and Section 2.2.7).

Additional Uncertainties - Data for humans exposed to low levels of these chemicals indicate that tetrachloroethylene inhibits trichloroethylene metabolism (Seiji et al. 1989). PBPK simulations of trichloroethylene and vinyl chloride indicate that competitive metabolic interactions between halogenated hydrocarbons only occur at high concentrations (Barton et al. 1995). Thus, tetrachloroethylene may inhibit trichloroethylene metabolism by a non-competitive mechanism. The design of the study observing joint action to increase serum ALT in rats (Stacey 1989) could not discern additive from greater-than-additive or less-than-additive joint action.

Table 17. Matrix of BINWOE Determinations for Nervous System Effects from Simultaneous Exposure to Chemicals of Concern

		ON TOXICITY OF			
		1,1,1-Trichloro-ethane	1,1-Dichloroethane	Trichloro-ethylene	Tetrachloro-ethylene
EFFECT OF	1,1,1-Trichloroethane		=IIC (0)	=IIC (0)	=IIC (0)
	1,1-Dichloroethane	=IIC (0)		=IIC (0)	=IIC (0)
	Trichloroethylene	=IIC (0)	=IIC (0)		=IIC (0)
	Tetrachloroethylene	=IIC (0)	=IIC (0)	=IIC (0)	

Condensed from ATSDR 2001a
(Numerical weight values are indicated in parentheses below)

INTERACTIONS: = additive (0); > greater than additive (+1); < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct & unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) & likely direction (0.71);
III: mechanistic data does not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A. toxicologic significance has been directly demonstrated (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0)
2: different exposure duration or sequence (0.79)
a: *in vivo* data (1.0)
b: *in vitro* data (0.79)
i: anticipated route of exposure (1.0)
ii different route of exposure (0.79)

Table 18. Matrix of BINWOE Determinations for Liver and Kidney Effects from Simultaneous Exposure to Chemicals of Concern

		ON TOXICITY OF			
		1,1,1-Trichloroethane	1,1-Dichloroethane	Trichloro-ethylene	Tetrachloro-ethylene
EFFECT OF	1,1,1-Trichloroethane		=IIC (0)	=IIB (0)	=IIB (0)
	1,1-Dichloroethane	=IIC (0)		=IIC (0)	=IIC (0)
	Trichloroethylene	=IIB (0)	=IIC (0)		=IIB (0)
	Tetrachloroethylene	=IIB (0)	=IIC (0)	<IIB (-0.50)	

Condensed from ATSDR 2001a
(Numerical weight values are indicated in parentheses below)

INTERACTIONS: = additive (0); > greater than additive (+1); < less than additive (-1); ? indeterminate (0)

MECHANISTIC UNDERSTANDING:
I: direct & unambiguous mechanistic data to support direction of interaction (1.0);
II: mechanistic data on related compounds to infer mechanism(s) & likely direction (0.71);
III: mechanistic data does not clearly indicate direction of interaction (0.32).
TOXICOLOGIC SIGNIFICANCE:
A. toxicologic significance has been directly demonstrated (1.0);
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals (0.71);
C: toxicologic significance of interaction is unclear (0.32).
MODIFYING FACTORS:
1: anticipated exposure duration and sequence (1.0)
2: different exposure duration or sequence (0.79)
a: *in vivo* data (1.0)
b: *in vitro* data (0.79)
i: anticipated route of exposure (1.0)
ii different route of exposure (0.79)