

Biotransformation of halogenated solvents

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ANDERS MW, JAKOBSON I. Biotransformation of halogenated solvents. *Scand J Work Environ Health* 11 (1985): suppl 1, 23—32. The toxicity of most halogenated solvents is associated with their biotransformation. The bioactivation can be due to the formation of stable, toxic metabolites or the formation of reactive, electrophilic intermediates. The paper summarizes present knowledge on the biotransformation of several halogenated compounds, ie, methyl halides, methylene halides, haloforms, carbon tetrachloride, chlorinated ethanes, and chlorinated alkenes. Some of the relevant enzymatic metabolic reactions are also discussed.

Key terms: bioactivation, carbon tetrachloride, chlorinated alkenes, chlorinated ethanes, chloroform, dichloromethane, hexachloro-1,3-butadiene, metabolism, methyl chloride, trichloroethylene.

Halogenated solvents constitute a large and heterogeneous group of halogenated hydrocarbons. Depending on their widespread use, as well as on their diversified chemical, physical, and biological properties, they are of utmost interest in occupational toxicology. Their environmental and metabolic stability varies from almost inert to highly reactive.

The toxicity of most halogenated hydrocarbons is associated with biotransformation to toxic metabolites. Often, the metabolism is accompanied by dehalogenation. The bioactivation of halogenated aliphatic hydrocarbons can be due to the formation of stable, toxic metabolites or to the formation of highly reactive intermediates. Examples of the former include the conversion of dihalomethanes (42) and haloforms (6, 74) to carbon monoxide and the formation of toxic mercaptans from methyl chloride (38). Examples of the formation of reactive metabolites include the conversion of chloroform to phosgene (62), of carbon tetrachloride to the trichloromethyl radical (13), epoxide formation from halogenated ethylenes (29), and the formation of episulfonium ions from *vic*-dihaloethanes (47).

This review summarizes current knowledge on the biotransformation of several important halogenated compounds and presents a brief discussion of some of the relevant enzymatic reactions. Several reviews on the toxicity and biotransformation of halogenated hydrocarbons are available elsewhere (3, 4, 11, 50, 60, 79).

Methyl halides

Methyl halides (monohalomethanes, CH_3X) are of great toxicologic interest, because they are used as fumigants, rather than as solvents. Both methyl iodide and methyl chloride form glutathione conjugates (33, 38), and the glutathione conjugate of methyl chloride *S*-methylglutathione may be metabolized to methanethiol. This occurrence is an example of a toxic activation after reaction with glutathione, and the reaction is probably catalyzed by cysteine conjugate β -lyase (38). This reaction pathway indicates a biotransformation mechanism completely different from those known for the polyhalogenated methanes. The neurotoxicity of methyl chloride (71) may be due to methanethiol formation (38). A proposed biotransformation scheme for methyl chloride is presented in figure 1. As shown in the scheme, formaldehyde and formate are other known metabolites.

Methylene halides

Methylene halides (dihalomethanes, CH_2X_2) are another toxicologically significant group of halogenated alkanes. Dichloromethane is particularly important because of its use as a solvent and degreaser and because of its role in the manufacture of decaffeinated coffee.

The metabolism of the dihalomethanes has been studied in detail, and two routes of metabolism have been identified, ie, the cytochrome P-450-dependent biotransformation to carbon monoxide and the glutathione *S*-transferase-dependent metabolism to formaldehyde and formic acid.

The mechanism of the metabolism of dihalomethanes to carbon monoxide has been elucidated earlier (39) and is illustrated in figure 2. The cytochrome P-450-dependent oxidation of dihalomethanes yields an unstable formyl halide intermediate, which may act as an acylating agent or may decompose to yield

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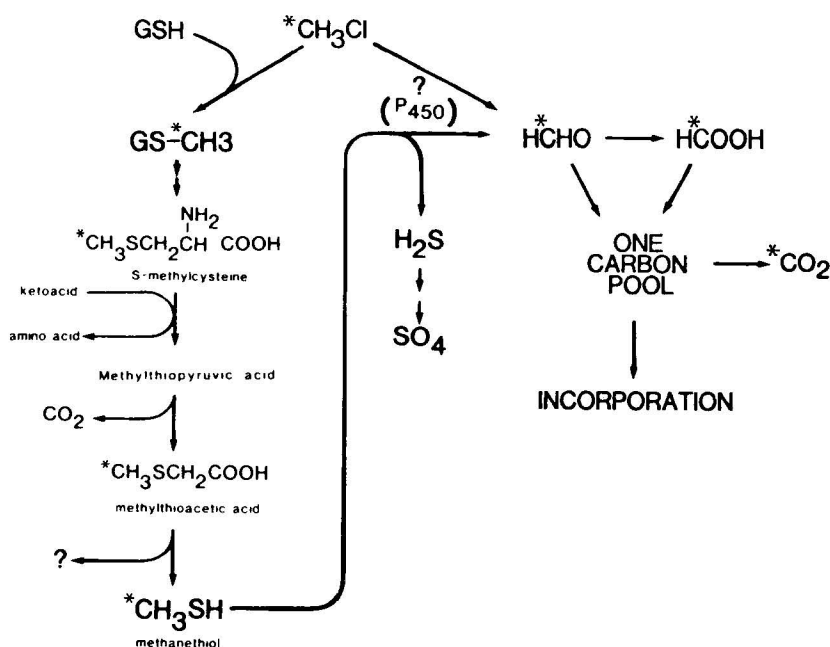


Figure 1. Proposed scheme for the metabolism of methyl chloride [reproduced from Kornbrust & Bus (38) with permission].

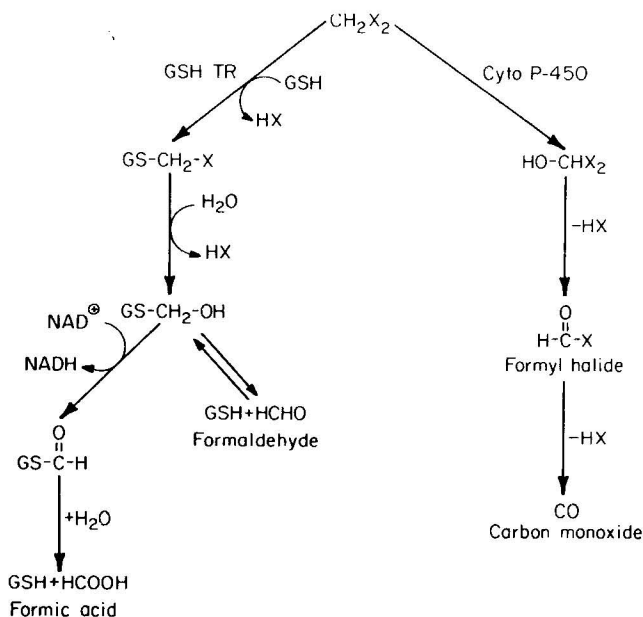


Figure 2. Glutathione S-transferase- and cytochrome P-450-dependent metabolism of dihalomethanes. (GSH TR = glutathione S-transferase, cyto P-450 = cytochrome P-450, GSH = glutathione)

carbon monoxide (5). The formation of carbon monoxide as a metabolite is toxicologically significant and has been associated with intoxications in man (75).

Reaction mechanism studies have shown that the glutathione S-transferase-dependent metabolism of dihalomethanes proceeds through an initial conjugation reaction to yield an S-halomethylglutathione intermediate, which undergoes hydrolysis to S-hydroxymethylglutathione (1) (figure 2). S-hydroxymethylglutathione is the hemimercaptal of formalde-

hyde and glutathione, and this reaction accounts for the formation of formaldehyde as a metabolite of dihalomethanes. Alternatively, S-hydroxymethylglutathione may be oxidized to S-formylglutathione by formaldehyde dehydrogenase, and S-formylglutathione may be hydrolyzed by S-formylglutathione hydrolase to yield formic acid.

The metabolism of dihalomethanes by glutathione conjugation or by microsomal oxidation is associated with the mutagenicity of dihalomethanes and may, therefore, be of toxicologic significance (35). The in-

terpretation of studies on the mutagenicity of dihalomethanes is, however, complicated by the observation that the bacteria used in the mutagenicity assay also metabolize dihalomethanes (22).

Haloforms

Haloforms (trihalomethanes, CHX_3) are important toxic chemicals. There is considerable low-level human exposure to chloroform formed during the disinfection of raw water supplies by chlorination. Occupational exposure to haloforms is also of importance, although their use as solvents has decreased because of their toxicity.

The metabolism of haloforms has been studied in several laboratories, and the major routes have been elucidated. The cytochrome P-450-dependent monooxygenase system catalyzes the metabolism of haloforms, and the metabolic pathway is summarized in figure 3. The reaction proceeds through the formation of a trihalomethanol, which loses HX to form a carbonyl halide, phosgene in the case of chloroform (62). The carbonyl halide can be trapped by reaction with cysteine to yield 2-oxothiazolidine-4-carboxylic acid. Hence it has been observed that cysteine inhibits the covalent binding of [^{14}C]chloroform metabolites to tissue proteins (66). In addition, [^2H]chloroform is metabolized to phosgene more slowly than chloroform (65). Toxicity studies show that cysteine administration decreases the toxicity of chloroform

(62) and that [^2H]chloroform is less toxic than chloroform (65). The available evidence about chloroform metabolism and toxicity suggests strongly that phosgene is the major reactive metabolite formed from chloroform.

Haloforms are also metabolized to carbon monoxide, an example of the formation of a stable, but toxic, metabolite of a halogenated chemical (74). In this case, the intermediate carbonyl halide is diverted to yield carbon monoxide in a thiol-dependent reaction.

Haloforms deplete the glutathione content of the liver. This occurrence is due to the reaction of glutathione with a carbonyl halide to form diglutathionyl dithiocarbonate as a metabolite (63).

Carbon tetrachloride

Carbon tetrachloride (CCl_4) is the only tetrahalomethane of practical toxicologic significance, and hence the discussion will be limited to this compound. The metabolism of carbon tetrachloride has been of interest for many years, because of the toxicologic importance of carbon tetrachloride and because of its frequent use as a model hepatotoxin.

The major pathways and metabolites of carbon tetrachloride are shown in figure 4. The available evidence suggests that the first step in the metabolism of carbon tetrachloride is a cytochrome P-450-catalyzed one-electron reduction to yield inorganic

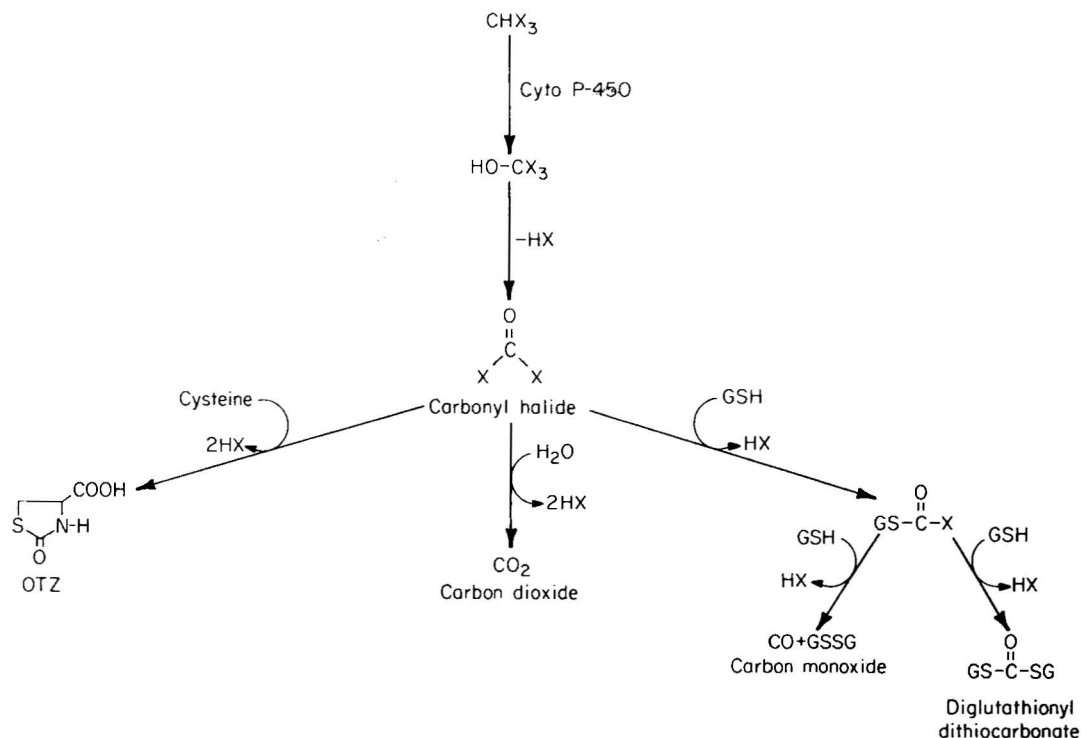


Figure 3. Biotransformation of haloforms. (Cyto P-450 = cytochrome P-450, GSH = glutathione, GSSG = glutathione disulfide, OTZ = 2-oxothiazolidine-4-carboxylic acid)

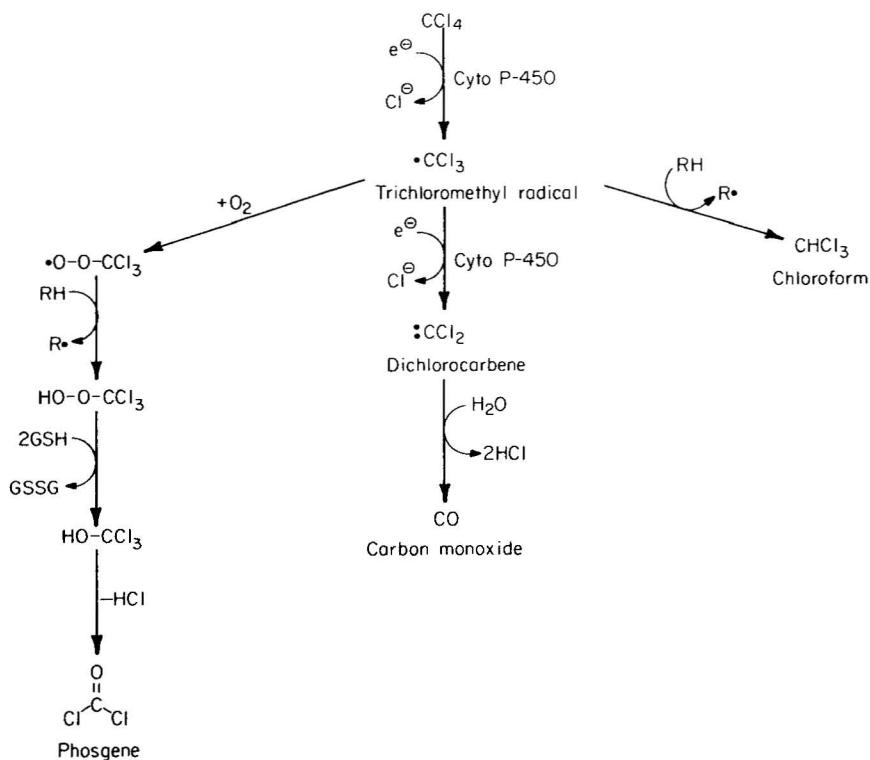


Figure 4. Biotransformation of carbon tetrachloride. (Cyto P-450 = cytochrome P-450, RH = unsaturated lipid, $\text{R}\cdot$ = lipid dieny radical, GSH = glutathione, GSSG = glutathione disulfide)

chloride and the trichloromethyl radical. The trichloromethyl radical is believed to play a central role in carbon tetrachloride metabolism, and all observed metabolites are presumably due to further reactions of the trichloromethyl radical.

The formation of the trichloromethyl radical, which was suggested in 1961 (13), has now been proved by electron spin resonance (ESR) studies. Although earlier results were controversial, ESR-spin trapping experiments with ^{13}C carbon tetrachloride as the substrate have shown conclusively that the trichloromethyl radical is a transient intermediate in carbon tetrachloride metabolism (51). Additional evidence for the formation of the trichloromethyl radical comes from the observation that 9- and 10-trichloromethylstearate are formed when carbon tetrachloride is incubated with a cytochrome P-450 system reconstituted in phospholipid vesicles (77); these adducts are formed by the reaction of the trichloromethyl radical with membrane lipids. Chloroform is a known metabolite of carbon tetrachloride (13). Reaction mechanism studies show that chloroform is formed as a consequence of hydrogen atom abstraction, presumably from microsomal lipids, by the trichloromethyl radical rather than by protonation of the trichloromethyl carbanion formed by a two-electron reduction of carbon tetrachloride (41).

The trichloromethyl radical may undergo a one-electron reduction to yield the trichloromethyl carbanion; the α elimination of chloride from the trichloromethyl carbanion yields dichlorocarbene,

which has been trapped and identified conclusively (64).

Finally, phosgene is a known metabolite of carbon tetrachloride (40, 73). Although the mechanism by which carbon tetrachloride is metabolized to phosgene is not fully understood, a "reductive-oxygenation" mechanism has been proposed in which the reaction of dioxygen with the trichloromethyl radical occurs followed by rearrangement to yield phosgene (67).

The toxicity of carbon tetrachloride is dependent on its metabolism. The formation of the trichloromethyl radical and its reaction with membrane lipids may initiate lipid peroxidation, which is a prominent biological event observed after carbon tetrachloride is given to animals (69). Phosgene is also a toxic, reactive metabolite of carbon tetrachloride and may play a role in carbon tetrachloride toxicity (26). Finally, studies on the synergistic toxicity of carbon tetrachloride and chloroform have led to the suggestion that the combined effects of phosgene formation and the initiation of lipid peroxidation may be involved in carbon tetrachloride toxicity (27).

Chlorinated ethanes

There are many chlorinated ethanes, but few are of current toxicologic importance. Hence the discussion focuses on 1,2-dichloroethane and 1,1,1- and 1,1,2-trichloroethane. The use of the last-mentioned

compound as a solvent is low because of its toxicity. Reviews of the metabolism and carcinogenicity of chlorinated ethanes have appeared recently (16, 49).

Cytochrome P-450-dependent oxygenation reactions are important in the metabolism of chlorinated ethanes, and glutathione *S*-transferase-catalyzed reactions are of toxicologic importance in the case of 1,2-dichloroethane. The metabolic pathways are summarized in figure 5.

The cytochrome P-450-catalyzed reactions yield an intermediate *gem*-chlorohydrin as the first product; such compounds are unstable and rearrange rapidly to give the corresponding aldehyde, which may be oxidized to a carboxylic acid or reduced to an alcohol. Thus 1,2-dichloroethane is metabolized to chloroacetaldehyde (24), and the major urinary excretion products are chloroacetic acid and metabolites derived from further metabolism of glutathione conjugates of chloroacetaldehyde (82). 1,1,2-Trichloroethane yields the same metabolites (81); this occurrence suggests that the initial hydroxylation reaction occurs on the carbon atom bearing two chlorines (32). In the case of 1,1,1-trichloroethane, the major metabolite is 2,2,2-trichloroethanol (32), which is excreted as the glucuronide conjugate (25). 2,2,2-Trichloroethanol excretion may afford a method for the biological monitoring of 1,1,1-trichloroethane exposure (15).

The glutathione *S*-transferase-dependent metabolism of 1,2-dichloroethane has been studied in

detail. Ethylene is a metabolite of 1,2-dichloroethane and is formed by a mechanism involving *S*-(2-chloroethyl)glutathione as an intermediate (46). Moreover, both the microsomal and cytosolic glutathione *S*-transferases catalyze the alkylation of deoxyribonucleic acid (DNA) by 1,2-dichloroethane metabolites (24). The putative alkylating species of 1,2-dichloroethane formed by the glutathione *S*-transferases is the episulfonium ion derived from *S*-(2-chloroethyl)glutathione. Finally, 1,2-dichloroethane is nephrotoxic, and recent studies suggest that *S*-(2-chloroethyl)cysteine, formed by peptidase action on *S*-(2-chloroethyl)glutathione, is involved (20).

Chlorinated alkenes

An important chemical and biotransformation reaction of halogenated alkenes is epoxidation. This primary biotransformation step often results in activation of the parent compound to a reactive intermediate, which may be involved in the toxicity.

The biotransformation of trichloroethylene to a reactive epoxide intermediate, ie, 2,2,3-trichlorooxirane, in humans was first proposed by Powell (68). Butler (12) suggested that trichloroethylene could be converted to chloral hydrate in the dog; the chloral hydrate is metabolized rapidly to trichloroethanol, which is partly excreted as a glucuronide (urochlorallic acid) and as trichloroacetic acid. Trichloroethanol,

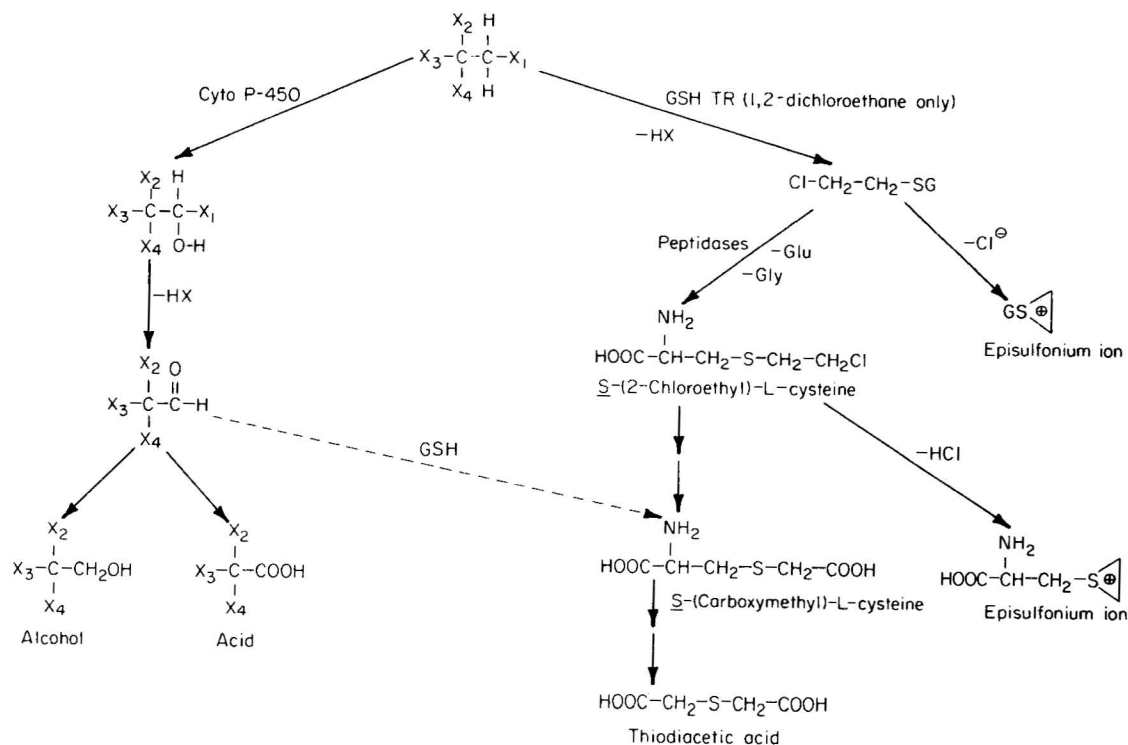


Figure 5. Biotransformation of chlorinated ethanes: 1,2-dichloroethane ($\text{X}_1 = \text{X}_2 = \text{Cl}$; $\text{X}_3 = \text{X}_4 = \text{H}$); 1,1,1-trichloroethane ($\text{X}_1 = \text{H}$; $\text{X}_2 = \text{X}_3 = \text{X}_4 = \text{Cl}$); 1,1,2-trichloroethane ($\text{X}_1 = \text{X}_2 = \text{X}_3 = \text{Cl}$; $\text{X}_4 = \text{H}$). (Cyto P-450 = cytochrome P-450, GSH TR = glutathione *S*-transferase)

urochloralic acid, and trichloroacetic acid are, indeed, well known urinary metabolites of trichloroethylene in man, as well as in many experimental animals (79). There are several *in vivo* studies on the metabolism of trichloroethylene which deal mainly with the quantification of these metabolites in rats (18, 36) and man (7, 21, 37, 57, 58).

In the metabolism of trichloroethylene, the migration of a chlorine atom from one carbon to another occurs. This migration process was studied in rats by Daniel (18), who showed that [^{36}Cl]trichloroethylene was biotransformed to [^{36}Cl]trichloroethanol and [^{36}Cl]trichloroacetic acid of the same specific activity, a result in accordance with the migration hypothesis. On the other hand, tetrachloroethylene underwent little metabolism in rat. The only identified urine metabolite was trichloroacetic acid, which could, however, be formed via an epoxide and by hydrolysis of trichloroacetyl chloride formed by a migration reaction similar to that suggested for trichloroethylene (18).

Leibman and a co-worker (14, 43) later showed that chloral hydrate was a metabolite of trichloroethylene in rat liver microsomes and that the reaction required reduced nicotinamide adenine dinucleotide phosphate (NADPH) and dioxygen. The formation of an intermediary epoxide was suggested.

Henschler and co-workers (10, 29) have studied the role of epoxide formation in the metabolism of halogenated alkenes in rat liver preparations, and they have also synthesized the chlorinated oxiranes expected as metabolites from several chloroethylenes (10, 29). The oxiranes give rise to either acyl chlorides (from tetra-, tri-, and 1,1-dichloroethylene) or to

aldehydes (from *E*- and *Z*-1,2-dichloroethylene) after thermal rearrangement. The acyl chlorides can be subsequently hydrolyzed to the corresponding acids, and the aldehydes can be oxidized to carboxylic acids or reduced to alcohols. The major metabolites of chloroethylenes are in accordance with those obtained in these studies, except in the case of trichloroethylene. Trichloroethanol and trichloroacetic acid (instead of dichloroacetic acid) are obtained *in vivo* after the rearrangement of trichloroethylene oxide (10, 29). Spectral evidence for the formation of 2,2,3-trichlorooxirane by microsomal oxidation of trichloroethylene with preparations from rabbit liver has been presented (78).

Dichloroacetic acid has also been identified in the urine of mice after intragastric trichloroethylene administration. This metabolic pathway might play an important role in the observed species differences in trichloroethylene toxicity, ie, a high mutagenic and tumor sensitivity in mice compared to low toxicity in rats and humans (28). Trichloroethylene also yields carbon monoxide *in vitro* (76), probably via an epoxide intermediate. However, carbon monoxide was not found *in vivo* in a study on mice and rats given [^{14}C]trichloroethylene intraperitoneally (61). On the other hand [^{14}C]carbon dioxide (5–15 % of the dose) was formed. In this study, a significant fraction of the radioactivity was bound to liver proteins, and some interaction with DNA was also observed.

Recently, an interspecies study on the biotransformation of trichloroethylene in rats and mice has presented evidence for a novel metabolite, ie, *N*-(hydroxyacetyl)-aminoethanol (19). This compound was

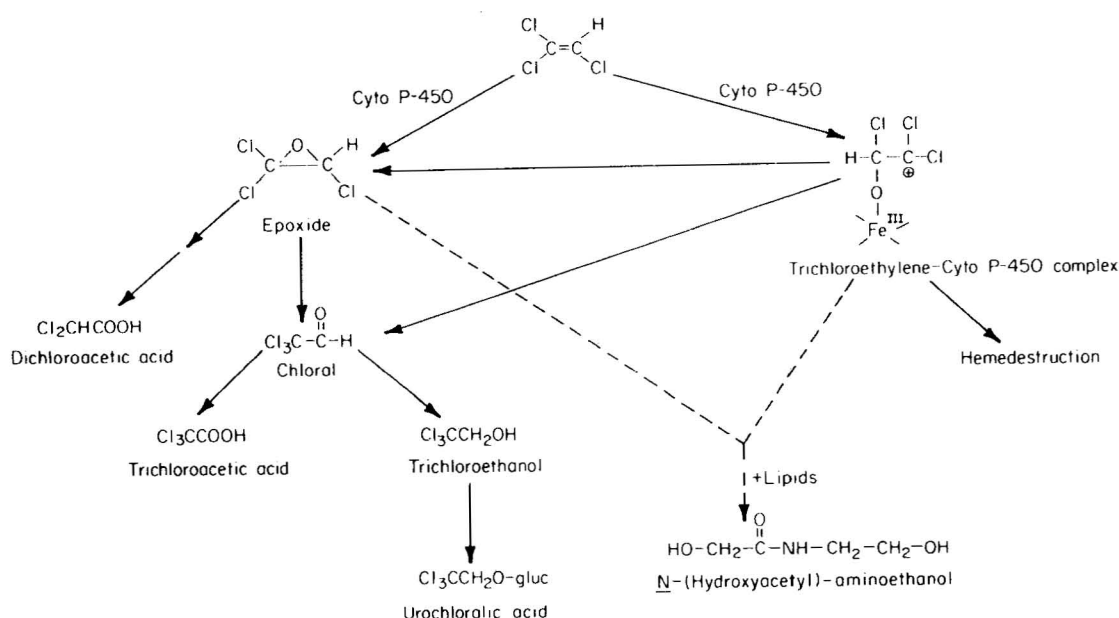


Figure 6. Biotransformation of trichloroethylene. (Cyto P-450 = cytochrome P-450, gluc = glucuronic acid)

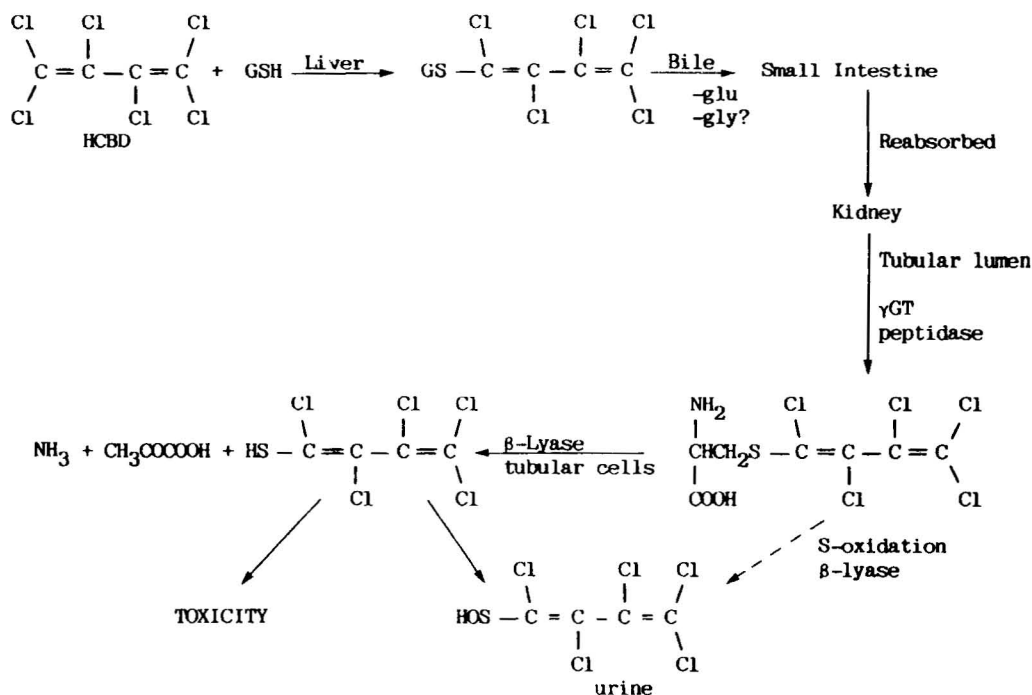


Figure 7. Proposed mechanism for hexachloro-1,3-butadiene-elicited nephrotoxicity [reproduced from Nash et al (59) with permission].

also found in the urine of humans exposed to trichloroethylene.

The asymmetrically substituted chlorinated ethylenes, ie, vinyl chloride, 1,1-dichloroethylene (vinylidene chloride), and trichloroethylene, are much more mutagenic than the symmetrically substituted compounds (tetrachloroethylene, *E*- and *Z*-1,2-dichloroethylene) (8, 23). This asymmetry increases the reactivity and electrophilicity of the chlorinated ethylenes and their corresponding oxiranes.

There are several indications supporting the view that epoxidation processes are of importance in the biotransformation of halogenated ethylenes. However, Miller & Guengerich (54, 55) have recently presented evidence that 2,2,3-trichlorooxirane is not an obligatory intermediate in the oxidation of trichloroethylene to chloral hydrate by cytochrome P-450. Instead, they proposed that the important chlorine migration reaction occurs in an oxygenated trichloroethylene-cytochrome P-450 transition state complex, yielding chloral and the other metabolites. These pathways are summarized in figure 6.

The oxidation of halogenated alkenes is catalyzed by cytochrome P-450-dependent monooxygenases, as evidenced by the subcellular location and cofactor requirements (30, 43, 54), by their response to enzyme inducers (44, 55, 56), by the spectral interactions with cytochrome P-450 (17, 30, 31, 72, 78), and by the finding of an inhibitory effect of carbon monoxide (2, 17).

1,1-Dichloroethylene is widely used in the manufacture of plastics. In contrast to trichloroethylene,

this compound is conjugated with liver glutathione to a great extent. Identified urinary metabolites include chloroacetic acid, thioglycolic acid, thiodiglycolic acid, dithioglycolic acid, and the mercapturic acids *N*-acetyl-S-(2-carboxymethyl)cysteine and *N*-acetyl-S-(2-hydroxyethyl)cysteine (34, 52, 53, 70). Furthermore, there are also indications of a completely different reaction mechanism, as shown by the formation of methylthioacetaminoethanol (70). Indeed, the formation of this compound has been suggested to explain, at least in part, the hepatotoxic effect of 1,1-dichloroethylene (70).

Hexachloro-1,3-butadiene, a by-product in the manufacture of tri- and tetrachloroethylene, is a potent nephrotoxic agent (48, 59). According to studies on rat liver and kidney preparations in vitro, hexachloro-1,3-butadiene is conjugated with glutathione; the reaction is catalyzed by the cytosolic and microsomal glutathione *S*-transferases (80). The glutathione conjugate is then further degraded, and cysteine conjugate β -lyase has been proposed to catalyze the formation of a sulfhydryl metabolite from the intermediary cysteine derivative (figure 7). The sulfhydryl metabolite could be responsible for the nephrotoxicity (80). The results have been confirmed by in vivo studies with male rats (59).

There are many reviews dealing with the biotransformation of halogenated alkenes (3, 4, 9, 45, 50). In several of these papers, the biotransformation of vinyl chloride is treated thoroughly, and it is, therefore, not discussed explicitly in this paper.

Acknowledgments

Research on the metabolism and toxicity of halogenated hydrocarbons conducted in the authors' laboratory was supported by NIEHS grants ES03126 and ES03127. Support to I Jakobson from the Swedish Work Environment Fund is gratefully acknowledged.

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