Metabolism of Halogenated Ethylenes
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The metabolism of the chlorinated ethylenes may be explained by the formation of chloroethylene epoxides as the first intermediate products. The evidence indicates that these epoxides rearrange with migration of chlorine to form chloroacetaldehydes and chloroacetyl chlorides. Thus, monochloroacetic acid, chloral hydrate, and trichloroacetic acid have been found in reaction mixtures of 1,1-dichloroethylene, trichloroethylene, and tetrachloroethylenes, respectively, with rat liver microsomal systems. Rearrangements of the chloroethylene glycols formed from the epoxides by hydration may also take place, but would appear, at least in the case of 1,1-dichloroethylene, to be quantitatively less important. The literature on the metabolism of chlorinated ethylenes and its relationship to their toxicity is reviewed.

Introduction
The demonstration that the carbon–carbon double bond in olefins (1–4), cyclic olefins (3), and aromatic compounds (5) are oxidized to epoxides by the mixed-function oxidases of mammalian liver microsomes has generated much interest in the metabolism of unsaturated compounds and its relationship to their toxicity. The epoxide ring tends to be chemically quite unstable, depending upon the configuration of the oxirane compound. It may thus react with a variety of groups in compounds of biological interest; when these compounds are essential to cellular function, such reaction may lead to alteration of cellular metabolism and subsequent cell necrosis, or to carcinogenic or mutagenic events.

Epoxides may be detoxified by hydration to diols, a reaction catalyzed by microsomal epoxide hydrase (6–8), or by reaction with soluble glutathione-epoxidetransferases with the ultimate production of mercapturic acids (9). Arene oxides may also rearrange to phenols (10).

Early studies with chlorinated olefins (11, 12) demonstrated the metabolic formation of products in which transfer of chlorine atoms from one carbon atom to another had taken place. The most likely pathway for such product formation would be via epoxidation of the double bond. The resulting halo-oxirane compounds are known to be extraordinarily unstable and rearrange spontaneously at rapid rates (13). Haloglycols that might be formed from such haloepoxides by enzymic or nonenzymic hydration would also be quite unstable, due to the presence of halogen and oxygen functions on the same carbon atoms, and would be expected to undergo rapid reactions analogous to the pinacol rearrangement. The recent discovery of the hepatotoxic, carcinogenic, and mutagenic potentials of a number of chlorinated ethylenes (14–18) has prompted new interest in the pathways of metabolism of these compounds.

We propose to review the literature on the metabolic transformations of the various chlorinated ethylenes, and to present in detail some of our own more recent work (19) on the products of metabolism of some of these compounds in vitro. We shall not discuss the metabolism of vinyl chloride, as this is the subject of several reports at this conference.

Trichloroethylene
Trichloroethylene was the first halogenated ethylene of which the metabolism was studied. After inhalation of this compound by dogs, the urine was found to contain trichloroacetic acid (20) and trichloroethanol (21), the latter both free and conjugated with glucuronic acid. These metabolites
were then shown to be excreted by humans and many experimental animals after exposure to trichloroethylene (22). One report exists of the excretion of small amounts of monochloroaetic acid by man after inhalation of trichloroethylene (23).

Butler (24) noted that the end products of metabolism of chloral hydrate and of trichloroethylene are identical, and he postulated (21) that chloral hydrate is an intermediate in the production of trichloroacetic acid and trichloroethanol from trichloroethylene. He was unable, however, to demonstrate the presence of chloral hydrate in dogs that had been exposed to trichloroethylene.

Proof for Butler's hypothesis was provided by the demonstration in our laboratory that the first stable, nonvolatile product of biotransformation of trichloroethylene in rat liver preparations is chloral hydrate. The identity of the product was established by a variety of chromatographic, colorimetric, chemical, and enzymic methods (12). The reaction was shown to be catalyzed by liver microsomes in the presence of a NADPH-generating system, and to behave as a typical mixed-function oxidase-mediated process (25). Formation of chloral hydrate from trichloroethylene was found to be stimulated in preparations from animals pretreated with phenobarbital or chronically exposed to trichloroethylene (26) and to be inhibited by metyrapone (27) and carbon monoxide (28). Furthermore, trichloroethylene produces a type I difference spectrum when added to liver microsomes (28, 29). The magnitude of type I binding of trichloroethylene is enhanced by pretreatment of animals with phenobarbital but not by that with 3-methylcholanthrene (29); chloral hydrate formation would therefore appear to involve cytochrome P-450 rather than cytochrome P-448.

The microsomal production of chloral hydrate from trichloroethylene in liver microsomes has been confirmed by Ikeda and Imamura (30), and chloral hydrate has been found in the plasma of rats (31) and humans (32) inhaling trichloroethylene, and to be produced in the isolated rat liver upon perfusion with trichloroethylene (13).

The enzymic pathway for metabolism of trichloroethylene was thus established, inasmuch as the enzymic mechanisms of conversion of chloral hydrate to trichloroethanol and trichloroaetic acid had already been demonstrated. The former process is catalyzed by liver alcohol dehydrogenase (33), whereas the oxidation of chloral hydrate to trichloroaetic acid is mediated by a NAD-requiring dehydrogenase in liver which is different from aldehyde dehydrogenase (34). That chloral hydrate is an intermediate in the formation of trichloroaetic ccid, trichloroethanol, and the glucuronide of the latter was confirmed by the demonstration that urinary excretion of these products by rats exposed to trichloroethylene was increased when the animals were pretreated with phenobarbital (26). Production of trichlorinated urinary metabolites is also enhanced after pretreatment with polychlorinated biphenyls, but not with 3-methylcholanthrene, hexachlorobenzene, spironolactone, or pregnenolone-16α-carbonitrile (18).

We therefore proposed the scheme shown in Eqs. (1) for the microsomal metabolism of trichloroethylene (12). In this hypothetical pathway, trichloroethylene is converted to its epoxide by the microsomal mixed-function oxidase system. The action of microsomal epoxide hydrase would convert this epoxide to trichloroethylene glycol. Rearrangement of the glycol by a reaction analogous to the pinacol rearrangement would produce chloral hydrate. Rearrangement of the epoxide with migration of chlorine would yield chloral, which would be hydrated immediately. If the epoxide were to rearrange with migration of a hydrogen atom, the product would be dichloroacetyl chloride, which would be hydrolyzed rapidly to dichloroaetic acid. It is of interest to note that the latter rearrangement is the one that normally occurs spontaneously in aqueous solutions, and that the presence of Lewis acids is required for the formation of chloral. By use of two gas-chromatographic systems (see below), we have been unable to detect the presence of dichloroaetic acid in extracts of incubation mixtures containing trichloroethylene, rat liver microsomes, and a NADPH-generating system, in which the formation of chloral hydrate could be demonstrated. Similarly, Bonse et al. (13) found chloral hydrate, but no dichloroaetic acid, after perfusion of isolated rat livers with trichloroethylene. It may be concluded that, if rearrangement of trichloroethylene oxide is involved in chloral hydrate formation, a directive influence of some microsomal component is exerted upon the ring opening.

Recent work on the metabolic activation of trichloroethylene also suggests that an epoxide is an intermediate in its metabolism. Van Duuren and Banerjee (35) have shown that after incubation of trichloro[14C]ethylene with rat liver microsomes in the presence of a NADPH-generating system, 14C...
becomes covalently bound to protein. This covalent binding was decreased when inhibitors of microsomal drug oxidation were present, and was increased in magnitude when the rats had been pretreated with phenobarbital, which enhances both the rate of metabolism (18, 26, 29) and the hepatotoxicity (18, 36) of trichloroethylene. The extent of covalent binding was diminished when various sulfhydryl compounds, which can react with epoxides, were present, and it was increased in the presence of 1,2-epoxy-3,3,3-trichloropropene, which is a known inhibitor of epoxide hydrolase. All the evidence thus far obtained, therefore, may be explained by the primary formation of trichloroethylene oxide, which may react with tissue constituents, rearrange to chloral, or be hydrated to a glycol.

Tetrachloroethylene

The next chlorinated ethylene for which metabolic studies were reported was tetrachloroethylene. In 1961, Yllner reported on the urinary metabolites of mice exposed to tetrachloro[14C]-ethylene (11). Of the total urinary radioactivity, 52% was present as trichloroacetic acid, 11% as oxalic acid, and a trace as dichloroacetic acid. No labeled monochloroacetic acid, formic acid, or trichloroethanol was found. However, 18% of the radioactivity was not extractable with ether, even after hydrolysis of the urine. Daniel (37) administered [38Cl] tetrachloroethylene orally to rats, and found only labeled trichloroacetic acid and inorganic chloride, in equimolar proportions, in the urine. These workers proposed that tetrachloroethylene oxide was the first product of metabolism, followed by rearrangement to trichloroacetyl chloride.

Trichloroacetic acid has since been observed to be a urinary metabolite of tetrachloroethylene in experimental animals and humans (18, 38-44). The excretion of total trichloro compounds, as measured by the Fujiwara colorimetric reaction after oxidation, exceeded that of trichloroacetic acid: in some cases this was assumed to be trichloroethanol (42, 43), whereas in other investigations it could not be demonstrated to be this compound (41). In one report, ethylene glycol was claimed to be a prominent metabolite (38).

By analogy with the metabolism of trichloroethylene, we have proposed (19) a scheme for possible pathways of metabolism of tetrachloroethylene [Eqs. (2)]. The primary reaction is hypothesized to be the formation of tetrachloroethylene oxide by mixed-function oxidation, followed by hydration of the oxide to trichloroethylene glycol. Because of

\[
\begin{align*}
\text{Cl}_2\text{C} &= \text{CCl}_2 \\
\text{Cl}_2\text{C} &= \text{CCl}_2 \\
\rightarrow \\
\text{Cl}_2\text{C} &= \text{COCl} \rightarrow \text{Cl}_2\text{C} \rightarrow \text{COOH}
\end{align*}
\]

(2)

the symmetry of these products, rearrangement of either would yield only one product, trichloroacetyl chloride, which would be hydrolyzed rapidly to trichloroacetic acid. To test this hypothesis, we have set up two gas-chromatographic systems that allow the separation and quantitation of mono-, di-, and trichloroacetic acids (Table 1). When extracts of incubation mixtures of tetrachloroethylene and rat liver 9000g supernatant fraction with a NADPH-generating system were analyzed by

### Table 1. Gas chromatographic retention times of possible acidic metabolites.

| Column       | Retention Times, sec |
|--------------|----------------------|
|              | MCA | DCA | TCA |
| Chromosorb 101 |     |     |     |
| 195°C        | 318 | 786 | 882 |
| 105°C        | 264 |     | 228 |

*Chromosorb 101 was used without coating. DC-560 was on Chromosorb P-AW-DMCS. Both were 80/100 mesh, in 1.8 m x 2 mm glass U-columns. For preparation of the trimethylsilyl (TMS) derivatives, 10 μl of pyridine was added to ether extracts, which were then dried over anhydrous MgSO₄, and the ether evaporated under a stream of nitrogen at room temperature. 50 μl of Pierce Tri-Sil was added, and 10 min later an aliquot was injected into the gas chromatograph. MCA, DCA, TCA denote mono-, di-, and trichloroacetic acids, respectively.

either method, a peak corresponding in retention time to that of trichloroacetic acid was obtained in an area of the chromatogram that was devoid of peaks in those of blank incubation mixtures to which tetrachloroethylene was not added until the end of the incubation, immediately before extraction. When the extracts were "spiked" with trichloroacetic acid, only one peak was observed. No mono- or dichloroacetic acid could be detected.

Trichloroacetic acid could also be quantitated by the sensitive modification of the Fujiwara alkaline pyridine method devised in our laboratory several years ago (45). When this method was used for the estimation of the trichloroacetic acid produced from tetrachloroethylene, the results were very similar to those obtained by the gas-chromatographic method (Table 2). The effects of various additives on the
Table 2. Formation of trichloroacetic acid from tetrachloroethylene in rat liver 9000g supernatant fraction.*

| Method               | TCA, nmol |
|----------------------|-----------|
| Gas chromatography   | 154       |
| Colorimetric assay   | 148       |

*Incubation mixtures contained, in a total of 5 ml, 100 μmole of nicotinamide, 3 μmole of ATP, 6 μmole of EDTA, 25 μmole of glucose 6-phosphate, 50 μmole of MgSO4, 0.3 μmole of NADP+ 98 μmole of tetrachloroethylene, 1 nmole of Tris buffer (pH 7.5), and reconstituted lyophilized 9000g supernatant fraction from 400 mg of liver from rats that had been pretreated with phenobarbital, 75 mg/kg, for 3 days. After incubation for 2 hr at 37°C, mixtures were chilled on ice for 10 min. For gas chromatography, the chilled mixtures were saturated with NaCl, adjusted to pH 1 with HCl, and extracted with 5 ml of ether; the extracts were evaporated to 100 μl at room temperature under a stream of dry nitrogen, and aliquots were injected into the gas chromatograph. For colorimetric analysis, the chilled incubation mixtures were deproteinized with 0.5 ml of 10% Na2WO4, and 0.5 ml of 2N H2SO4, and aliquots of the supernatant liquid were subjected to procedure A of the modified Fujiwara reaction (45).

reaction are shown in Table 3. EDTA, ATP, and nicotinamide all had small enhancing effects when magnesium ions were present. Surprisingly, magnesium ion inhibited trichloroacetic acid production at a concentration (10 mM) that enhances most microsomal oxidations, including the production of chloral hydrate from trichloroethylene (25). In the absence of magnesium, EDTA had no significant effect, whereas ATP and nicotinamide still en-

Table 3. Production of trichloroacetic acid from tetrachloroethylene in rat liver 9000g supernatant fraction.

| System                             | % of control |
|------------------------------------|--------------|
| Complete, with Mg++               | 100          |
| less EDTA                          | 73           |
| less ATP                           | 83           |
| less nicotinamide                  | 75           |
| less Mg++                          | 186          |
| Complete, without Mg++             | 100          |
| less EDTA                          | 106          |
| less ATP                           | 79           |
| less nicotinamide                  | 78           |

The complete system as described in Table 2.

hanced the reaction rate slightly. Table 4 shows that the nucleotide-generating system was required for production of trichloroacetic acid from tetrachloroethylene; NADH substituted very poorly for NADPH, and no synergism was observed when the two reduced nucleotides were present together. Finally, Table 5 shows that when cyclohexene was added to the incubation mixture to inhibit epoxide hydrase, no difference in trichloroacetic acid formation was observed. It may be concluded that, if the epoxide-diol pathway is operative, either trichloroethylene oxide is not subject to hydration by epoxide hydrase, or else both the epoxide and the glycol are rearranged to trichloroacetyl chloride at similar rates.

Bosse et al. (13) also found trichloroacetic acid to be the only detectable metabolite in isolated rat liver perfused with tetrachloroethylene. Trichloroacetic acid was found free in the perfusate, and could also be extracted from the liver after vigorous acid hydrolysis. They postulated that trichloroacetyl chloride reacted with cell constituents, resulting in acylation. This may be the cause of the hepatotoxicity of tetrachloroethylene noted by Moslen et al. (18). They showed that pretreatment with polychlorinated biphenyls increased both the production of trichloroacetic acid and the level of serum transaminase after exposure to tetrachloroethylene. Greim et al., however, did not find tetrachloroethylene to be mutagenic to bacteria after incubation with liver microsomes and a NADPH-generating system (16).

Table 4. Coenzyme requirement for conversion of tetrachloroethylene to trichloroacetic acid in rat liver microsomes.*

| Generating system | TCA formed, nmol |
|-------------------|------------------|
| None              | 0                |
| NADPH             | 80.1             |
| NADH              | 5.5              |
| NADPH + NADH      | 86.9             |

*Incubation conditions were similar to those of Table 2, except that microsomes from 400 mg of liver were used. For the NADPH-generating system, 2 units of glucose 6-phosphate dehydrogenase were added. For the NADH-generating system, NADP+ was replaced by an equal quantity of NAD+, glucose 6-phosphate by 1 μl of ethanol, and glucose 6-phosphate dehydrogenase by 2 units of alcohol dehydrogenase.

Table 5. Effect of epoxide hydrase inhibition on production of trichloroacetic acid from tetrachloroethylene.*

| Cyclohexene oxide | TCA formation, % of control |
|-------------------|-----------------------------|
| M                  |                             |
| 5 × 10^-5         | 96                          |
| 5 × 10^-4         | 107                         |
| 5 × 10^-3         | 81                          |

*Incubation conditions as described in Table 2.

1,1-Dichloroethylene

The metabolism of 1,1-dichloroethylene (vinylidene chloride) had apparently not been studied prior to 1975 (46). By analogy to the metabolism of trichloroethylene, we postulated the pathway (19) shown in Eqs. (3). If the epoxide-diol pathway is

![Chemical Structure]

(3)

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operative, rearrangement of the glycol, or of the epoxide with migration of a chlorine atom, should give rise to monochloroacetyl chloride, which would then be hydrolyzed to monochloroacetic acid. Rearrangement of the epoxide with migration of a hydrogen atom would yield dichloroacetaldehyde. We were able to demonstrate the presence of monochloroacetic acid in extracts of incubation mixtures of 1,1-dichloroethylene with rat liver 9000g supernatant fraction and a NADPH-generating system by both of the above-mentioned gas-chromatographic systems. No dichloroacetic acid was detected. However, another peak was present in the chromatogram of the underivatized extract, which as yet is unidentified; we have not been successful in obtaining a satisfactory gas chromatogram from samples of dichloroacetaldehyde freshly prepared from its acetal.

As seen in Table 6, the production of monochloroacetic acid from 1,1-dichloroethylene is extensively inhibited by magnesium ion. EDTA enhanced the reaction in the presence or absence of magnesium: ATP was required in the presence but not the absence of magnesium, whereas the opposite was the case with nicotinamide. The significance of these observations is not clear at present.

Table 6. Production of monochloroacetic acid from 1,1-dichloroethylene in rat liver 9000g supernatant fraction.*

| System | % of control |
|--------|-------------|
| Complete, with Mg** | 100 |
| less EDTA | 14 |
| less ATP | 0 |
| less nicotinamide | 124 |
| less Mg** | 1800 |
| Complete, without Mg** | 100 |
| less EDTA | 14 |
| less ATP | 96 |
| less nicotinamide | 23 |

*In incubation conditions were similar to those of Table 2, except that tetrachloroethylene was replaced by 132 μmole of 1,1-dichloroethylene.

Table 7. Coenzyme requirement for conversion of 1,1-dichloroethylene to monochloroacetic acid in rat liver microsomes.*

| Generating system | MCA formed, nmole |
|-------------------|-------------------|
| None | 0 |
| NADPH | 856 |
| NADH | 0 |
| NADPH + NADH | 880 |

*In incubation conditions were similar to those of Table 4, except that tetrachloroethylene was replaced by 132 μmole of 1,1-dichloroethylene.

hydrase by either cyclohexene oxide or 1,2-epoxy-3,3,3-trichloropropane results in stimulation of the formation of monochloroacetic acid to about double the control value; higher concentrations of the inhibitors apparently interfere with the epoxidation reaction. We may conclude that the larger part of the monochloroacetic acid is formed by rearrangement of the epoxide, and that the glycol is relatively unimportant as a source of monochloroacetic acid. Inhibition of the conversion of the epoxide to the glycol therefore allows the accumulation of the epoxide and a greater rate of production of monochloroacetic acid from the epoxide.

The relationship of the metabolism of 1,1-dichloroethylene to its toxicity is not clear. The mutagenicity of this compound to bacteria in the presence of liver microsomes requires a NADPH-generating system and is increased when the rodent donors of the microsomes are pretreated with phenobarbital (47). However, pretreatment with phenobarbital or polychlorinated biphenyls has been reported to protect experimental animals from the hepatotoxicity caused by 1,1-dichloroethylene (48, 49). Unlike the case of vinyl chloride, the hepatotoxicity of which shows a positive correlation with hepatic microsomal cytochrome P-450 content, the hepatotoxicity of 1,1-dichloroethylene shows a negative correlation with cytochrome P-450 levels (49). Jaeger et al. have shown that the hepatotoxicity of 1,1-dichloroethylene is greatest under conditions in which liver glutathione levels are lowest (15, 50, 51). If this hepatotoxicity is due, not to the epoxide metabolite but to 1,1-dichloroethylene per se, a reaction between glutathione and the chloroethylene may be catalyzed by glutathione S-alkenotransferase (9). Alternatively, differential induction of microsomal mixed-function oxidase and epoxide hydrase activities by phenobarbital may greatly increase the diol/epoxide ratio in this case, which would reduce the hepatotoxicity if this were caused by the epoxide. As noted above, hydration of 1,1-dichloroethylene oxide would appear to be subject to inhibition; it would be interesting to test the effect of these inhibitors on the hepatotoxicity of 1,1-dichloroethylene.

Table 8. Effect of epoxide hydrase inhibition on production of monochloroacetic acid from 1,1-dichloroethylene.*

| Inhibitor | Concentration, M  | % of control |
|-----------|------------------|-------------|
| Cyclohexene oxide | 5 × 10^-4 111 |
| | 5 × 10^-3 209 |
| | 3 × 10^-2 102 |
| 1,2-Epoxo-3,3,3-trichloropropane | 5 × 10^-5 107 |
| | 5 × 10^-4 200 |
| | 5 × 10^-3 181 |

*Conditions were as in Table 6.

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1,2-Dichloroethylene

A scheme for the metabolism of 1,2-dichloroethylene, analogous to those presented above, is shown in Eqs. (4). One might expect the production of dichloroacetaldehyde by rearrangement of the glycol or of the epoxide with migration of a chlorine atom, or of monochloroacetyl chloride, and therefore monochloroacetic acid, by rearrangement with migration of a hydrogen atom. We have found no significant amounts of monochloroacetic acid in extracts of incubation mixtures of either cis- or trans-1,2-dichloroethylene with rat liver 9000g supernatant fraction and a NADPH-generating system. A chromatographic peak was obtained from experiments with either isomer that was similar in retention time to the unknown peak obtained in experiments with 1,1-dichloroethylene. Attempts to identify this peak as dichloroacetaldehyde have been inconclusive.

Bonse et al. (13) have demonstrated the production of small quantities of dichloroacetic acid and of dichloroethanol in the isolated rat liver perfused with either cis- or trans-1,2-dichloroethylene. By analogy with the metabolism of trichloroethylene, this may indicate the initial formation of dichloroacetaldehyde.

In summary, it has been demonstrated that di-, tri-, and tetrachloroethylenes are converted in liver microsomes to products that may be explained by assuming that the first reaction is the formation of an epoxide bridge at the double bond, and that the resulting chloroethylene oxide rearranges, with migration of a chlorine atom, to yield an aldehyde or acyl halide. Rearrangement of the glycol formed from the epoxide by hydration may also be involved but, at least in the case of 1,1-dichloroethylene, this reaction would appear to be quantitatively less important.

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