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Metabolism and Mutagenicity of Halogenated Olefins—A Comparison of Structure and Activity

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Chlorinated ethylenes are metabolized in mammals, as a first step, to epoxides. The fate of these electrophilic intermediates may be reaction with nucleophiles (alkylation), hydrolysis, or intramolecular rearrangement. The latter reaction has been studied in the whole series of chlorinated epoxiethanes. The rearrangement products found were: acyl chlorides (tetrachloro-, trichloro-, and 1,1-dichloroethylenes), or chlorinated aldehydes (1,2-dichloroethylenes, *cis*- and *trans*-, vinyl chloride). The metabolites found *in vivo* are identical with, or further derivatives of these rearrangement products, with one important exception: trichloroethylene. With this compound, *in vivo* rearrangement yields chloral exclusively. The mechanism of the different rearrangement has been identified as a Lewis acid catalysis.

All chlorinated ethylenes have been investigated in a tissue-mediated mutagenicity testing system. The prominent molecular feature of those with mutagenic effects (trichloro-, 1,1-dichloro-, and monochloroethylene) is unsymmetric chlorine substitution which renders the epoxides unstable, whereas symmetric substitution confers relative stability and nonmutagenic property.

Since vinyl chloride has been demonstrated carcinogenic in humans (*1*) and experimental animals (*2*), and further reports suggested carcinogenic properties of the chemical congeners vinylidene chloride (*3, 4*) and trichloroethylene (*5*), we became interested in the question whether or not other members of the group of chlorinated ethylenes would share this type of biological activity, and what would be the molecular features or prerequisites, respectively, which are responsible for the carcinogenic effect. We started with a theoretical consideration of the chemical activity of the molecules which focused on the influence of the chlorine substitutions. In a second step, the metabolic bioactivation and degradation was studied *in vivo* and compared with the theoretical expectations from the chemical reactivity. After this, the mutagenic activity was investigated *in vitro* in a modified Ames testing system. Finally, the results of this mutagenicity test were compared with the *in vivo* carcinogenicity findings as reported by others, and a tentative rule of chemical reactivity, biotransformation and mutagenicity/carcinogenicity was worked out.

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Chemical Reactivity

The chemical reactivity of chlorinated aliphatic compounds is decisively determined by the chlorine substitution(s). However, the influence is completely different in alkanes, alkenes, and alkynes. In general, chlorine substitution exerts a stabilizing effect on account of steric protection by the bulky substituent. This interferes with the electron-withdrawal effect of the chlorine substituent. In the case of chlorinated alkanes, the result is a destabilization of C-C and C-Cl bonds. C-C and C-Cl fissions under formation of free radicals are consequent reactions in the metabolic conversions of C_1 or C_2 compounds.

A quite different influence is encountered with chlorinated ethylenes. In this class of molecules, the electron-withdrawal effect dominates over the mesomeric donator effect of the involved carbon atom, thus decreasing the electron density in the double bond which in turn results in a chemical stabilization against electrophilic attacks (*6*). The stabilizing effect increases with the number of chlorine substitutions. It is optimal in tetrachloroethylene, as has convincingly been demonstrated by the reactivity of chlorinated ethylenes with ozone (*7*).

In alkynes, chlorination results in a destabilization, as has been shown with mono- and dichloroacetylene, which are considerably less stable than acetylene (*8*).

Metabolic Activation and Deactivation of Chlorinated Ethylenes

The first step of metabolic transformation of chlorinated ethylenes in mammals is epoxidation. Up to now there is only indirect evidence for this reaction, (a) by the demonstration of a specific binding spectrum with P-450 in the case of trichloroethylene (*9*), and (b) by the type of metabolites identified *in vitro* as well as *in vivo*.

In general, chlorinated epoxides may undergo a variety of reactions (see Fig. 1): reaction with nucleophilic cellular macromolecules under alkylation; conjugation with low molecular nucleophiles (mainly glutathione) both enzymatically and nonenzymatically; hydrolysis to diols, again with and without the catalytic action of enzymes (epoxide hydrase); and intramolecular rearrangement. The latter reaction represents a deactivation mechanism and is of considerable importance for the potential of acute toxicity as well as of carcinogenicity and mutagenicity of the different members of the series of chlorinated ethylenes.

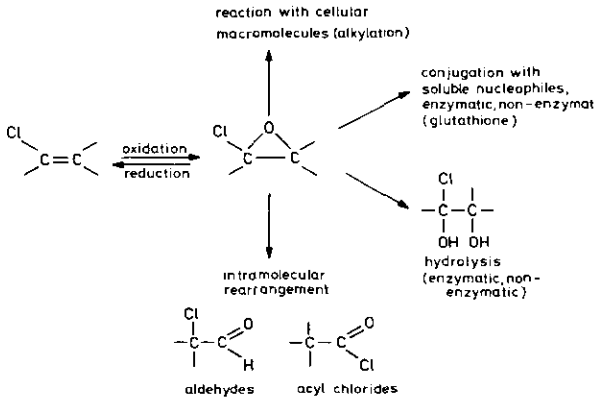
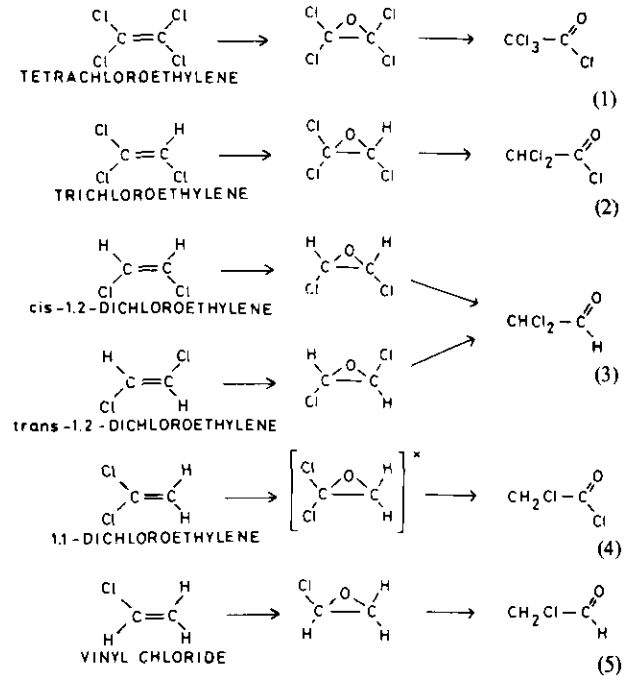


FIGURE 1. Reactions of chlorinated aliphatic epoxides after formation in the cell.

We have studied the chemical behavior of these epoxides which have been synthesized. The exception was 1,1-dichloroethylene oxide, which cannot be obtained by conventional methods, apparently because it is extremely unstable on account of its high polarity (*10*). They rearrange either to acyl chlorides (tetrachloro-, trichloro-, and 1,1-dichloroethylene) or to chlorinated aldehydes *cis*- and *trans*-dichloroethylene, and vinyl chloride) [Eqs. (1)-(5)]. The expected metabolites would be trichloroacetic acid (with tetrachloroethylene), dichloroacetic acid (tri- and 1,2-*cis*- and *trans*-dichloroethylene) or monochloroacetic acid (vinylidene and vinyl chloride), and derivatives of monochloroacetic acid (or chloroacetaldehyde) after conjugation with glutathione.

The predicted metabolites have been identified in systematic experiments with the isolated perfused rat liver preparation (*10*), with one important exception: trichloroethylene. With this compound, essentially no dichloroacetic acid was detected; this has recently been confirmed by Leibman in a microsomal metabolizing system (*11*). The different behavior of trichloroethylene oxide *in vitro* (thermal rearrangement to dichloroacetyl chloride) and *in vivo* (formation of chloral) is outlined in Figure 2.



This difference might be of crucial significance for the evaluation of the carcinogenic potential of trichloroethylene. We therefore studied the rearrangement mechanisms in a more detailed investigation. Theoretically, there are three paths of intramolecular rearrangement. A simple hydride migration which would lead to dichloroacetyl chloride has low probability of occurring (*12*). The formation of α-ketocarbonium ions after C-O heterolysis which is the most probable path, could occur in two versions, resulting either in a single [Eq.

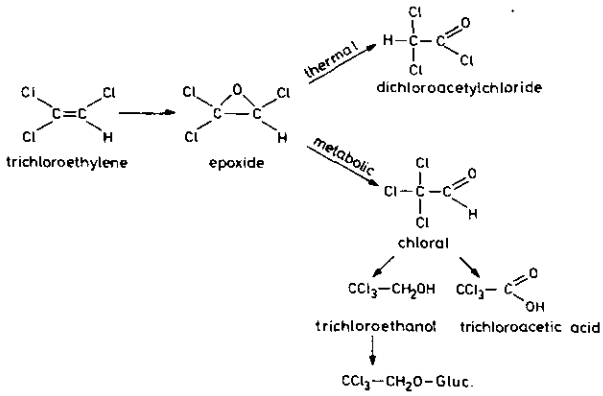
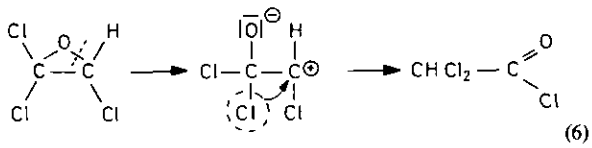


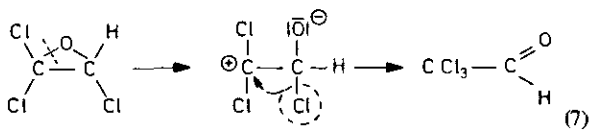
FIGURE 2. Formation of trichloroethylene epoxide and rearrangement *in vitro* (to dichloroacetyl chloride) and *in vivo* (to chloral) and further metabolic products.

(6)] or a double chlorine-substituted carbenium [Eq. (7)]; the latter (disubstituted), which would yield chloral, is less stable and thus has the lower probability.

The rearrangement to chloral [Eq. (7)], which is the only path detected *in vivo* can be forced *in vitro* by the catalytic action of strong Lewis acids like $FeCl_3$ or $AlCl_3$ (*10*). We have speculated therefore, that the only formation of chloral (and further oxidation or reduction products such as trichloroacetic acid and trichloroethanol, and its glucuronide) *in vivo* could be due to catalytic action of the iron of P-450 in the trivalent form at the site of the formation of the epoxide (*6*); experiments with purified P-450 to confirm or reject this assumption are in progress.



(6)



(7)

Mutagenicity of Chlorinated Ethylenes

The six chlorinated ethylenes have been tested for their mutagenic potential in a modified Ames tissue-mediated microbial system (*13*). *Salmonella typhimurium* as tester strains, which had previously been successfully used with vinyl and vinylidene chloride (*14, 15*), were found unsuitable because of the high primary toxicity exerted by some of the compounds. After extended preliminary studies, *E. coli* K 12 was found an acceptable tester strain.

The results are given in Figure 3. There was no direct mutagenic activity of the compounds. Only after metabolic activation by added phenobarbital-induced mouse microsomes some of the compounds yielded positive results. The data cannot be compared on a quantitative basis because the concentrations of the test compounds varied from 0.6*mM* (tetrachloroethylene) to 10.6*mM* (vinyl chloride). However, vinyl chloride obviously is the most active compound. Vinylidene chloride and trichloroethylene exert a small but definite mutagenic effect, whereas tetrachloroethylene and 1,2-*cis*- and *trans*-dichloroethylenes are inactive.

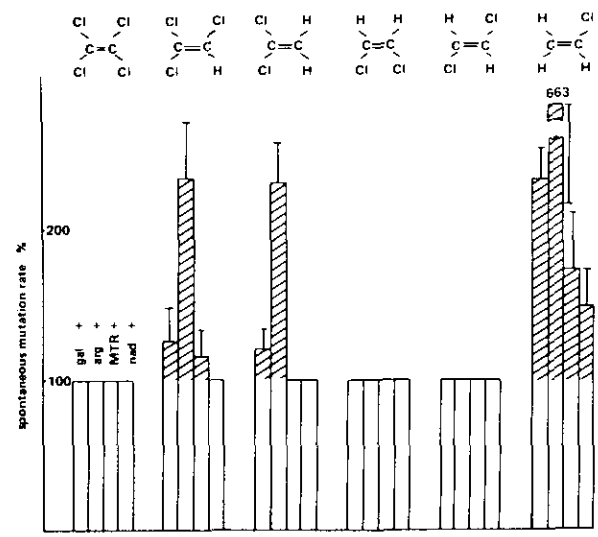


FIGURE 3. Mutagenicity of chlorinated ethylenes in *E. coli* K 12 after metabolic activation by incubation with stimulated mouse liver microsomes (*13*).

Chemical Reactivity of Metabolically Formed Epoxides; Mutagenicity and Carcinogenicity

A view at the synopsis of the structural peculiarities of the epoxides (Fig. 4) reveals that the common feature of those which are mutagenic is an unsymmetric chlorine substitution, whereas the inactive compounds are characterized by a symmetric distribution of the chlorine atoms. There is evidence from the chemical literature that unsymmetric chlorine substitution renders the epoxides unstable (*10, 16*), as compared to symmetric substitution, where the epoxides are relatively stable (*17, 18*). The reason for the instability of an unsymmetric substituted oxirane is the preponderance of the electron withdrawal effect of the chlorine atom(s).

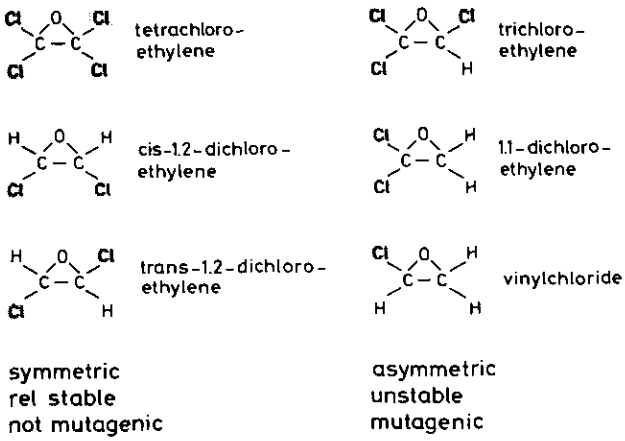


FIGURE 4. Tentative rule of interrelationship between chemical structure, electrophilicity (stability), and mutagenic (carcinogenic) potential of epoxides of chlorinated ethylenes (*19*).

We have deduced from these interrelationships the following tentative rule: high electrophilicity is a prerequisite for the mutagenic and carcinogenic activity of the unsymmetric chlorinated ethylenes trichloro-, 1,1-dichloro-, and monochloroethylene (*13, 19*). So far, the predicted carcinogenicity has been confirmed by vinyl chloride (*1, 2*), vinylidene chloride (*3, 4*), and trichloroethylene (*5*).

However, the carcinogenic potential of trichloroethylene has not yet been demonstrated convincingly. The NCI study (*5*) has been performed with a technical sample which contained strong electrophilic epoxides as stabilizers (epichlorohydrin, epoxibutane) (*20*) which most probably confer the carcinogenic effect. As pointed out above, the chemical behavior of trichloroethylene oxide differs *in vitro* and *in vivo*. The probability prevails that this epoxide is completely detoxified by rearrangement to chloral under *in vivo* conditions in mammals. A solution of this problem is open to further carcinogenicity tests in whole animals and biochemical studies on the bioactivation and deactivation of trichloroethylene under realistic exposure conditions.

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