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METABOLISM AND RELATIVE CARCINOGENIC POTENCY OF CHLOROETHYLENES: A QUANTUM CHEMICAL STRUCTURE-ACTIVITY STUDY

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SUMMARY

Properties of six chloroethylenes which could serve as indicators of their relative metabolic behavior and carcinogenic activity have been calculated using Modified Neglect of Diatomic Overlap (MNDO), a semiempirical, all valence electron, molecular orbital method. Possible pathways of transformation of parent compounds to acylchlorides, chloroaldehydes and epoxides — their putative ultimate carcinogens — were considered, and heats of formation and relative stabilities of intermediates were calculated. Our results indicate that carbonyl compounds could be formed with and without the intermediacy of epoxides, suggesting the possibility of more than one pathway in activation of parent compounds. Electronic properties of carbonyl products and epoxide carbocations, putative ultimate carcinogens which could serve as indicators of their relative electrophilicities, were also calculated. The results obtained indicated that the relative extent of metabolism to carbonyl products, rather than their electrophilicity, is a determinant of the relative carcinogenic activity of the parent compound. Of the various thermodynamic criteria investigated, four were found to be indicators of both relative metabolic behavior and carcinogenic activity.

Key words: Carcinogenicity — Chloroethylenes — Quantum chemical — Structure activity — Vinylchloride

Abbreviations: LUMO, lowest unoccupied molecular orbital; MNDO, Modified Neglect of Diatomic Overlap; UHF, Unrestricted Hartree-Fock.

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INTRODUCTION

The ethylene chlorides are widely used in industry as degreasers, solvents and cleaning fluids. Recent interest in these compounds has been generated by the observations that vinyl chloride and some of its analogs cause cancer in laboratory animals [1–3]. Vinyl chloride is potentially harmful to humans as well. Studies of workers who handle vinyl chloride have shown serious increase in expected tumor incidence and mortality [4,5]. Also of significance is the finding that these compounds fail in the Ames mutagenicity test. This test shows the ethylene chlorides to be only weakly mutagenic even though they are carcinogens [6].

Research on vinyl chloride (monochloroethylene) has shown that, like many carcinogens, it does not directly cause cancer. Instead, it is metabolized to reactive electrophilic intermediates which act as the ultimate carcinogens [7]. This has been found to be true for the more highly chlorinated ethylenes as well [8].

Despite extensive studies of two prototype compounds, vinyl chloride and vinylidene chloride (1,1-dichloroethylene), many questions regarding the mode of action of haloethylenes as chemical carcinogens and the properties which determine their relative activity remain unresolved. For example, it is not clear whether epoxides or acylchlorides and chloroaldehydes are the ultimate carcinogens. In addition, the mode of formation of chloroaldehydes and acylchlorides from parent compounds and the involvement of the epoxides as intermediates are not clear. Finally, the relative importance of metabolism and reactivity of ultimate carcinogens with tissue macromolecules in determining carcinogenic activity of parent compounds has not been established.

In this study we have used the techniques of theoretical chemistry to investigate properties of the six ethylene chloride compounds and their intermediates which could be reliable indicators of their extent of biotransformation to active carcinogens and of the ease of adduct formation of the putative ultimate carcinogen with nucleic acid components. Because these compounds require biotransformation to reactive ultimate carcinogens, these two types of properties should be important in determining the relative carcinogenic potency of the parent compounds. If such properties could be identified and calculated, they would serve two purposes: (1) further elucidation of the mechanism of chemical carcinogenicity of unsaturated aliphatic halohydrocarbons; (2) provide a basis for development of a rapid screening procedure for predicting at least the presence or absence of activity in unknown compounds of this type.

In previous work we have performed such mechanistic structure-activity studies of polycyclic aromatic hydrocarbons and their amine and methyl derivatives and were able to identify reliable molecular indicators of relative metabolism and carcinogenic activity [9]. The selection of such indicators was based on existing knowledge of the mechanisms of biotransformations, leading to the ultimate carcinogens in a given class of compounds and of the

interactions of the ultimate carcinogens with tissue macromolecules that could lead to carcinoma.

While there are many unknown aspects of the transformation of unsaturated halohydrocarbons to their active forms and the role of competing detoxification pathways, direct evidence for the involvement of cytochrome *P*-450 in the oxidation of vinyl chloride and vinylidene chloride is substantial [10—13]. Less is known about the more highly chlorinated ethylenes and the role of *P*-450 here can only be inferred. Indirect evidence, however, supports this hypothesis [14—16].

As shown in Fig. 1, the first step in enzymatic transformation of the chloroethylenes is generally thought to be oxidation by cytochrome *P*-450 by asymmetric addition of a radical oxene species to the carbon-carbon double bond of the substrate [17,18]. A radical mechanism is favored by studies of isotope effects on rates of reaction [19] and by the 'suicide substrate' action of vinyl chloride. During the biotransformation of vinyl chloride by *P*-450, a reactive species is formed that can react with the heme group and destroy the enzyme [20]. A radical intermediate can potentially cause this destruction. This 'suicide substrate' action is also suspected for other chloroethylenes [21]. Our own previous mechanistic studies also favor a radical mechanism [22,23].

The biradical formed can then either isomerize by H or Cl atom shifts, forming stable acylchlorides and aldehydes or ring close to form an epoxide. Further, if the epoxides are exposed to aqueous environment, there is strong evidence that they isomerize to acylchlorides and chloroaldehydes by H^+ and Cl^- 1,2 shifts [24].

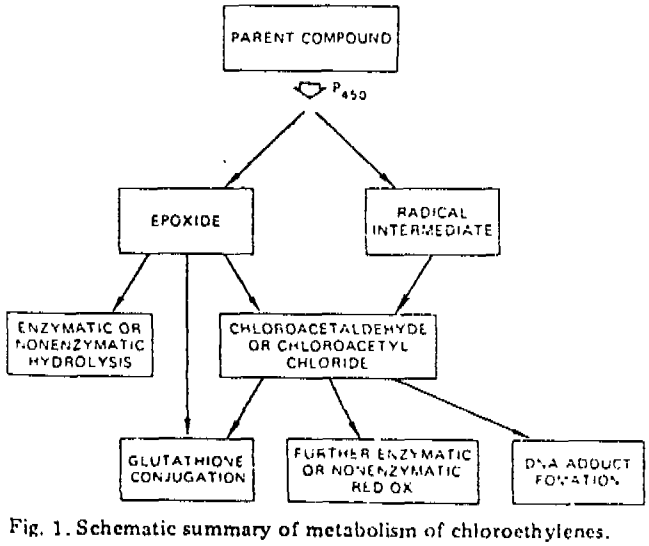


Fig. 1. Schematic summary of metabolism of chloroethylenes.

Alternatively, epoxides can undergo enzymatic or non-enzymatic hydrolysis. Some evidence for hydrolysis products of trichloroethylene and tetrachloroethylene epoxide exist; chloral hydrate has been reported as a metabolic product of trichloroethylene and oxalic acid as the tetrachloroethylene metabolite [12,14,25]. However, the relative amounts of these products and whether their formation is a direct result of hydrolysis is uncertain.

Finally, other enzymes such as glutathione *S*-transferase have been implicated in either activation or detoxification processes [26–28] involving epoxide or carbonyl compounds, but evidence for their involvement is much less clear than that for *P*-450. Oxidation and reduction of the aldehydes to alcohols and acids are known to occur [29] and these products are among the major metabolites found.

There is substantial evidence that the ultimate carcinogens are the acylchlorides or aldehydes [20,30–32]. However, the epoxides have also been implicated [20,30–32]. Both the chloroepoxides and aldehydes are electrophilic enough to form adducts with nucleophilic sites of DNA, and evidence for such adducts comes from extensive in vitro and in vivo studies with vinyl chloride [30,31,33,34]. Two types of DNA adducts, shown in Fig. 2, have been identified, Type 1 from in vitro and in vivo studies with vinyl chloride and DNA from a variety of species [29–31] and Type 2 most recently found in animals exposed to vinyl chloride [34]. As shown in Fig. 2, the nature of these adducts provides direct evidence that chloroaldehyde is the adduct-forming metabolite. However, as also shown in this figure, the N_7-adduct 2 could also be formed by a carbocation of the epoxide which attacks N_7 with loss of HCl from the adjacent carbon. If formation of such adducts is important in tumor formation, then acylchlorides and chloroaldehydes are likely active forms of ethylene chlorides, with or without the intermediacy of epoxides.

It should be pointed out that DNA alkylation is not the only proposed mechanism for chemical carcinogenesis. One proposed alternative is an 'epigenetic' mechanism. According to this hypothesis, widespread alkylation of macromolecules by the reactive intermediates causes destruction of tissue. The increased DNA synthesis required to make new tissue would be accompanied by an increase in spontaneous mutations and thus an increase in tumor formation [35]. While some recent evidence favours this mechanism for tetrachloroethylene [35], more work is needed to establish its relevance. In general the bulk of evidence points to a more specific effect on tissue macromolecules involving an electrophilic intermediate as the ultimate carcinogen, a criterion which is fulfilled by the chloroepoxide or carbonyl products.

Table I presents a summary of the relevant metabolic data. For all chloroethylenes, the oxidized and reduced products of the chloroacetaldehyde and chloroacetylchloride derivatives have been identified as metabolites. There have been no in vivo studies done involving identification of metabolites of *cis*- and *trans*-1,2-dichloroethylene. However, preliminary studies on perfused rat livers show small amounts of dichloroacetic acid

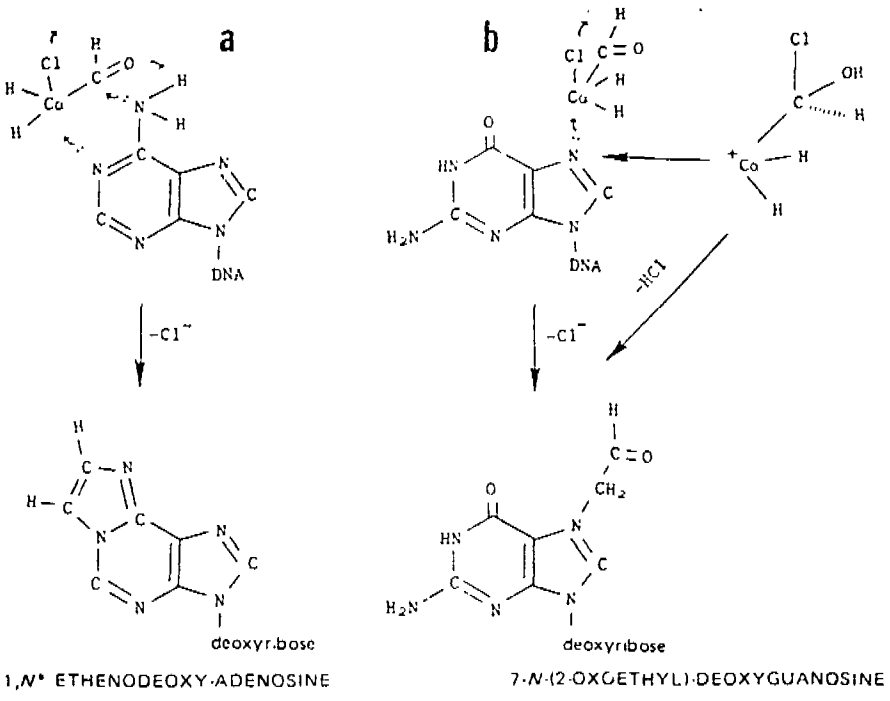


Fig. 2. Identified adducts of vinyl chloride metabolites with adenosine and guanosine.

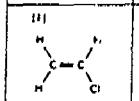
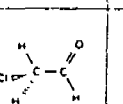
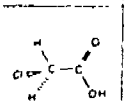
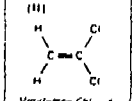
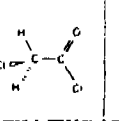
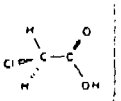
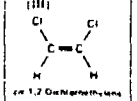
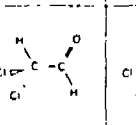
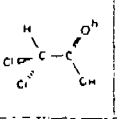
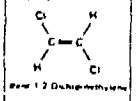
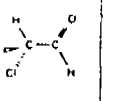
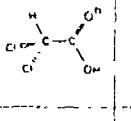
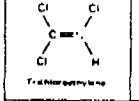
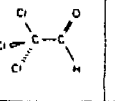
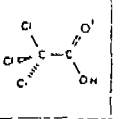
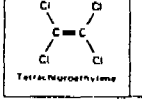
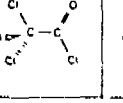
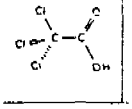
produced [24]. Table I also shows percentage unchanged parent compound recovered after in vivo metabolism. The values shown are from a variety of studies [16,24,26,27,36–42]. From the diverse studies reported, the percentage of unchanged parent compound appears to depend on factors such as dose, species of animal used and perhaps route of administration as well as details of methodology. There were gaps in many of these studies and only one investigated all six parent compounds [36].

The results of all these studies indicate that the extent of metabolism decreases generally with increasing degree of halogen substitution. The rank order of metabolism is: vinyl chloride ~ vinylidene chloride > *trans*-1,2-dichloroethylene ~ *cis*-dichloroethylene > trichloroethylene > tetrachloroethylene, with one inhalation study in rats [36] allowing us to place *cis*- and *trans*-1,2-dichloroethylene in this rank order.

Quantitative conclusions about the carcinogenic potency of these compounds cannot be made at this time due to lack of available data. Data available in the literature allows formulation of, at best, a tentative rank order of carcinogenic potency : vinyl chloride > vinylidene chloride > tetrachloroethylene > trichloroethylene, with not enough information available

TABLE I

SUMMARY OF THE OBSERVED PRODUCTS AND EXTENT OF OXIDATIVE METABOLISM OF CHLOROLTHYLENES

	Parent	Intermediate acylchloride/ chloroacetaldehyde	Final observed product	% Original compd[a] recovered when administered in vivo
K-1711	(I)  Vinyl chloride			1–4[b] 1[d]
K-2197	(II)  Vinylidene Chloride			1–2[c] 15[d]
K-1388	(III)  cis-1,2-Dichloroethylene			20[d]
K-1387	(IV)  trans-1,2-Dichloroethylene			4[d]
K-2520	(V)  Trichloroethylene			80–90[e] 36[f] 27[f] 8[d]
K-2521	(VI)  Tetrachloroethylene			70–98[g] 7[d]

[a] Percent of unchanged parent compound depends on four factors: (1) Dose (higher dose, greater % unchanged). (2) Species, e.g., rat, mice, human; in general, metabolism greater in mice than rats. (3) Route of administration, i.e., inhalation/oral; though studies are conflicting on this point. (4) Detailed methodology used.

[b] Range of percent unchanged; 1–2% oral administration, 0.05—1 mg/kg in male rats [26], 2% inhalation 10 ppm male rats [37], 4% by intragastric administration 250 mg/kg [42]. But note 67% unchanged in oral administration of 100 mg/kg in male rats [26].

[c] < 1% Intragastric administration of 350 mg/kg in male rats [27].

[d] Calculated concentration of parent compound after 9 h in the tissue compartment on exposure of male rats by inhalation to a constant level of 100 ppm, relative to vinyl chloride [36].

[e] 80—90% Unchanged in oral administration in rats [38].

[f] 27% Unchanged in inhalation studies of humans [39].

[g] 70% Unchanged by *oral* and *inhalation* studies in rats [40]. 98% Unchanged by oral administration in rats and 82% in mice [38].

[h] Products detected in metabolic studies of perfused liver [24] and liver microsomes [41].

[i] Some trichloroethanol is also observed.

OXIDATIVE

to place *cis*- and *trans*-1,2-dichloroethylene in this order. As with relative metabolism, carcinogenic potency roughly decreases with increasing degree of halogen substitution.

Though many sources were consulted, our rank order is based mainly on work done by C. Maltoni and coworkers [1–3,43–45] and by the National Cancer Institute [46,47]. The order VC > VDC was made on the basis of reported inhalation studies and the order tetra- > trichloroethylene on the basis of the NCI feeding and gavage bioassays. The laboratory of Dr. Bruce Ames has also used the NCI bioassays to rank tetra- > trichloroethylene in carcinogenic activity in female mice as part of their comprehensive project to select reliable carcinogenic studies from the literature and represent carcinogenic activity in quantitative form. In one study, vinylidene chloride and tri- and tetrachloroethylene were evaluated in mice [48] and this study was used to rank vinylidene chloride > tri- or tetrachloroethylenes, allowing the tentative ranking of the four compounds given above. This rank order is very tentative due to several problems in evaluating these data. No study has been done which includes all six chloroethylenes. Different routes of administration and different dose ranges made comparison indirect. Also, some compounds show species specificity. For example, tetrachloroethylene is carcinogenic in mice but not in rats. In general, the rank order was obtained from results with female mice, which were the most susceptible species.

To select fundamental molecular properties which might be reliable indicators of the relative metabolism and carcinogenic activity of these compounds, the following assumptions were made: (1) *P*-450 oxidation is the initial step of transformation leading to the active carcinogenic forms. (2) Acylchlorides and chloroaldehydes can be formed directly from radical products of *P*-450 oxidation or *via* an epoxide intermediate in an aqueous environment. (3) Acylchlorides and chloroaldehydes are candidate active carcinogenic forms which act as electrophiles in forming adducts with nucleophilic sites of DNA bases. (4) Carbocations formed from protonated epoxides could also be active carcinogenic forms which can form adducts with DNA if they are prevented from facile rearrangement to aldehydes. (5) Properties related to extent of metabolism of the parent compound to the active form and the electrophilicity of the putative ultimate carcinogen could be reliable indicators of their relative carcinogenic behavior.

In addition to these general assumptions, to select properties relevant to extent of metabolism of parent compounds, we have used three possible pathways for oxidative metabolism of the chlorinated ethylenes to active carcinogens formulated from existing experimental and theoretical evidence; these are shown in Fig. 3. The initial step (a) common to all subsequent transformations is *P*-450-mediated addition of triplet oxygen across the carbon-carbon double bond yielding a triplet biradical. We propose that there are then two possible fates for this biradical. In Pathway I, the initial triplet radical directly isomerizes via a chlorine or hydrogen atom 1, 2 shift (Ib). The rearranged triplet can then undergo intersystem crossing (Ic) to form the product carbonyl compound. Thus, in Pathway I, formation of

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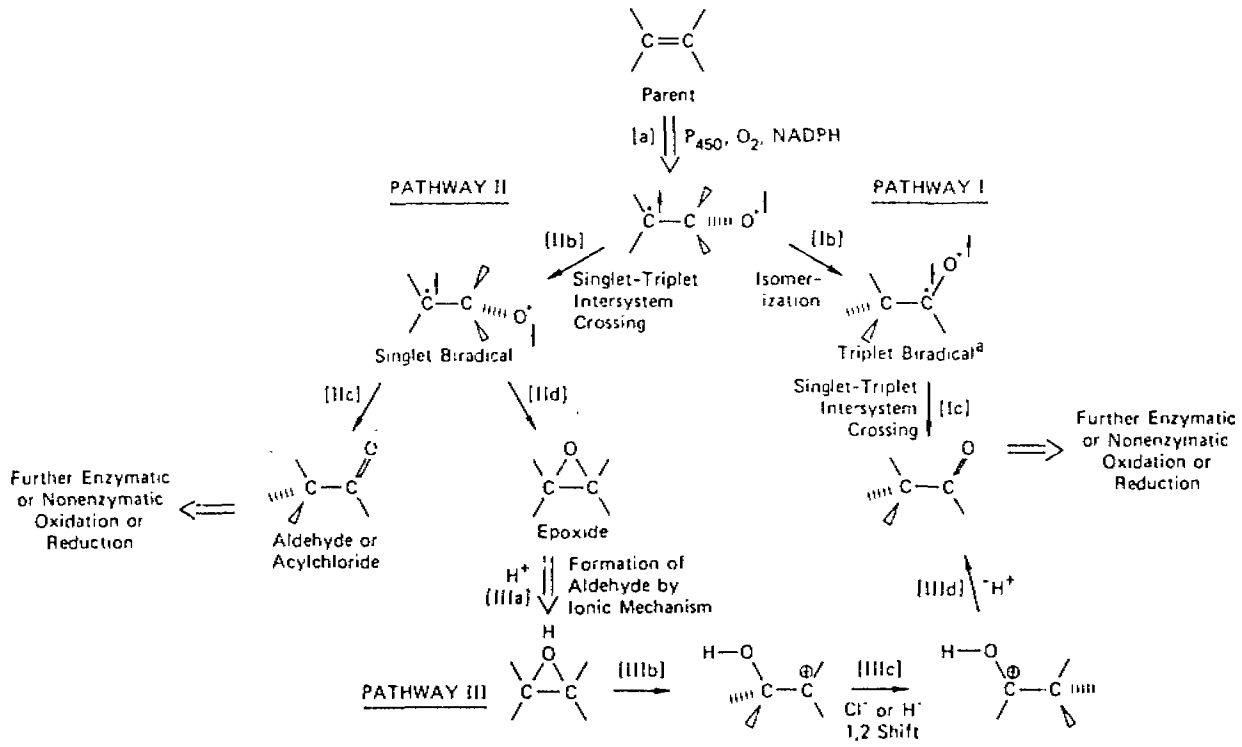


[a]Biradical from a Cl· or H· 1,2 shift in triplet state.

Fig. 3. Three postulated pathways to formation of epoxide and carbonyl products from metabolism of chloroethylenes.

acylchlorides and chloroaldehydes and their oxidation and reduction products does not proceed through an epoxide intermediate. However, in an alternate path (Pathway II), the initial triplet radical can undergo singlet-triplet intersystem crossing (IIb) and the resultant singlet biradical species can isomerize to form the putative carbonyl ultimate carcinogen (IIc) or can close to form an intermediate epoxide (IId). The epoxide could then be further transformed to chloroaldehydes in solution (Pathway III), providing an alternate, ionic pathway to formation of the aldehyde products. In this proposed pathway, the epoxide ring is protonated (IIIa) and the ring opens (IIIb). The carbocations formed undergo 1, 2 H^- or Cl^- ion shifts (IIIc) similar to those involving radical species and the rearranged product loses a proton (IIId) to form the carbonyl compound.

In our studies we have calculated enthalpies of reaction for each of these postulated mechanisms (Pathways I, II and III of Fig. 3) as possible indicators of their relative extent of total metabolism. By comparing calculated values of enthalpies of reaction corresponding to each pathway with known metabolic behavior, it was possible to identify those values which are the most reliable indicators of relative extent of metabolism to known products and thus to further elucidate the mechanisms of biotransformation of the parent chloroethylenes to carbonyl products.

As discussed, the nature of the two types of adducts isolated and identified from vinyl chloride administration in vivo and in vitro (Fig. 2) most directly implicates the carbonyl compounds as the active carcinogens. However, it is possible that the carbocation of the epoxide formed by ring opening of the protonated epoxide (Step IIIb, Fig. 3) could also be the ultimate carcinogen. While this possibility is less likely due to the facile rearrangement of these carbocations to aldehydes, these carbocations are also considered as putative ultimate carcinogens in this study. Properties of both types of putative ultimate carcinogens, aldehydes and epoxides, which could serve as measures of their electrophilicity and hence ease of formation of known adducts with nucleic acid bases were also calculated.

Among these two classes of properties, reliable indicators of relative carcinogenic activity were identified which could be useful in screening for carcinogenic activity in related untested compounds.

These results suggest that, while there may be other activating pathways, the *P*-450-mitigated ones chosen here are important. Further experimental tests of the reliability of the molecular indicators of activity identified here would provide additional verification of the importance of the underlying mechanism upon which they are based.

1,2 Shift

[a]Biradical from a Cl^- or H^- 1,2 shift in triplet state

Fig. 3. Three postulated pathways to formation of epoxide and carbonyl products from metabolism of chloroethylenes.

CHOICE OF MOLECULAR INDICATORS OF RELATIVE CARCINOGENIC ACTIVITY

(1) Properties relative to extent of transformation of chloroethylenes to active forms

Differences in calculated heats of formation $\Delta(\Delta H_f)$ among various

species in the three proposed pathways to the ultimate carcinogen were used as indicators of the relative extent of metabolism of each of the six chloroethylenes to their active form. These properties are related only to electronic factors which can influence the extent of transformation of parent compounds. The effect of transport or steric factors at the enzyme active site were not included. All of the species for which heats of formation were calculated are minima (i.e., reactants, intermediates or products), rather than transition states in each reaction pathway. For example, minima in Pathway I are: parent compound, triplet radical, isomerized triplet radical and the carbonyl product. The calculated difference in enthalpy of formation between two minima is related to an enthalpy of reaction rather than an enthalpy of activation for each step. Thus thermodynamic, rather than kinetic, criteria are used as a measure of relative extent of transformation of parent compounds. Such thermodynamic criteria are appropriate if each reaction is presumed to reach equilibrium. All $\Delta(\Delta H_f)$'s for each step in the reactions were calculated for each proposed pathway.

Specifically, as a measure of the relative extent of the initial *P*-450 oxidation of each parent compound, common to formation of both aldehydes and epoxides from parent compounds (Step a, Fig. 3), the stability of the proposed radical intermediate relative to the parent compound was used:

$$\Delta(\Delta H_f)_{\text{triplet-parent}} = \Delta H_f(\text{triplet radical}) - \Delta H_f(\text{parent})$$

Then, following Pathway I to formation of aldehydes, heats of formation were calculated for all geometry optimized isomerized triplet biradicals and their carbonyl products. The radicals result from 1, 2 shifts of H and Cl atom in each initial triplet radical.

Calculated heats of formation of the isomerized radicals were then used to calculate relative isomerization energies:

$$\Delta(\Delta H_f)_{\text{isomer}} = \Delta H_f(\text{isomerized triplet}) - \Delta H_f(\text{initial triplet})$$

and to calculate the stability of the carbonyl products formed from them:

$$\Delta(\Delta H_f)_{\text{product radical}} = \Delta H_f(\text{carbonyl product}) - \Delta H_f(\text{isomerized triplet radical})$$

Similarly, following Pathway II to formation of epoxides, as illustrated in Fig. 3, additional heats of formation were calculated for optimized geometries of the singlet biradical and the epoxides of each parent compound, and differences in all relevant heats of formation calculated.

As indicated in Pathway III, epoxides can also be transformed to carbonyl products by a non-enzymatic ionic mechanism. To model the energetics of this reaction pathway, we have calculated the heats of formation of the optimized geometries of (1) the protonated epoxides, (2) each carbocation

formed by ring opening of the protonated epoxide and (3) isomerized carbocation resulting from a 1, 2 H^- and Cl^- shift of each initial carbocation. Differences in heats of formation between these species and the carbonyl products were used as measures of the relative extent of such product formation via an epoxide intermediate.

Calculated values of all thermodynamic quantities were compared with the relative extent of metabolism and rank order of carcinogenic activity for all parent compounds.

(2) Electrophilic properties of putative active carcinogens

Two types of properties were chosen as indicators of electrophilic activity of acylchlorides and chloroaldehydes, one relevant to electrophilicity in incipient covalent bond formation and the other to more long-range electrostatic interactions [49—51].

In incipient covalent bond formation, the lowest unoccupied molecular orbital (LUMO) of the electrophile functions as the acceptor of electron density from the attacking nucleophilic centers of the DNA bases. The energy of this orbital was thus chosen as a good indicator of electron transfer. In addition, the more electron density the two carbon atoms have in this orbital, the more they function as localized electrophilic centers in covalent bond formation.

Electron affinities, i.e., the energy gained in adding an electron to the neutral form of each carbonyl product, could also have been calculated. However, since the reaction with DNA bases to form adducts does not involve addition of an electron, i.e., reduction of the carbonyl compounds, but simply partial electron donation and covalent bond formation, there is no reason to believe that electron affinities would be a better indication of electrophilicity of the neutral compound than its E_{LUMO}.

The net charges on the two carbon atoms were chosen as monitors of their electrostatic electrophilicity. The larger the positive charge on these atoms, the greater their long range attraction to the electron-rich nitrogen atoms of the DNA bases.

An additional requirement could be that the C_α carbon must have at least one halogen atom, since in an S_{N_1} displacement, a good leaving group like Cl^- facilitates attachment of the incoming nucleophile.

To examine the possibility that carbocations of epoxides could also be active carcinogenic forms of the ethylene chlorides, the energy of LUMO and the net charge on the cationic carbon atom of these species were also tabulated.

These calculated electrophilic properties of the putative ultimate carcinogens, together with those that indicate the extent of metabolism of the parent compounds, provided a set of molecular properties which could be used as indicators of rank order of carcinogenic activity of the chloroethylenes.

METHODS AND PROCEDURE

All calculations made in this study were performed using an all-valence semiempirical molecular orbital method called MNDO introduced in 1977 by Dewar and Thiel [52—54]. The method is parameterized to yield reliable optimized molecular geometries for species which have a finite lifetime, i.e., a minimum in their potential energy surfaces and the corresponding heat of formation of such species at 298°C. Using the MNDO method, then, optimized geometries and heats of formation were calculated for the parent chloroethylenes and all intermediates and products in the proposed pathways to their transformations to active forms indicated in Fig. 3. Total geometry optimization of radical species was performed using a MNDO Unrestricted Hartree-Fock (UHF) formalism [55] suitable for species with unpaired electrons; heats of formation, spin densities and electron densities were calculated. This method underestimates the individual heats of formation of radicals by as much as 20 kcal/mol. However, errors tend to cancel in comparisons made for a series of similar radicals [53]; it is only such comparisons which are made in this study.

Input geometries for MNDO calculations of parent chloroethylenes and product chloroacetaldehydes and chloroacetylchlorides were taken from experimental data [56—58]. Full geometry optimizations were performed. These involved optimization with respect to all geometric quantities: bond lengths, bond angles, and torsion angles. The criteria used for convergence was a maximum value ≤ 1 kcal/Å for the sum of the squares of the gradient components [52]. All parent compounds and products converged to well-defined optimized geometries.

In contrast to the parent compounds and final products, there are no structural data available for the chloroethylene oxides, since they are very unstable. Input geometries were constructed from a combination of crystal structure data for ethylene oxide and standard bond lengths and angles [57,59]. All epoxides converged to well-defined optimized configurations.

The proposed triplet and singlet biradicals and carbocations are too reactive to have been isolated. Therefore, no experimental structural data are available for them. However, previous theoretical studies have been done for some analogs [53] and input geometries were constructed from results of these studies as well as from standard bond length and bond angle values [57]. MNDO optimized geometries of the triplet biradicals were used as input for the singlet biradical calculations. All triplet biradicals converged to well-defined optimized geometries. However, one singlet biradical, $H_2C \cdot \uparrow\!-\!CHClO \cdot \downarrow$, did not fully converge by our criteria. The estimated error in the value of ΔH_f obtained is ±0.003 kcal/mol, too small to influence the significance of the results. Similarly, as noted in Table VIII, two carbocations did not quite converge on optimized geometries but were also estimated to have a very small residual error.

RESULTS

Tables II--IV present a comparison of MNDO calculated optimized geometries for parent compounds, intermediate epoxides and aldehydes, respectively, with geometries obtained by using a minimum basis set (STO-3G) ab initio method [60,61] and from experimental data when available. All other calculated geometries can be obtained upon request.

A comparison of MNDO optimized geometries for parent chloroethylenes with values obtained using the STO-3G ab initio method [61] and with experimental data [56—59] (Table II) shows that the MNDO method yields reliable geometries with all bond lengths within 0.03 Å and bond angles within 3° of experimental results. The greatest discrepancies in both MNDO and STO-3G are the C-Cl bond lengths. MNDO overestimates these bond lengths by about 0.02 Å, and STO-3G by 0.05 Å. Where comparisons of bond angles can be made, both methods are accurate to about 2°.

The calculated and experimental values [62,63] for the dipole moments are also shown in Table II. Both methods overestimate the dipole moments. However, MNDO's values are substantially better than those obtained by the minimum basis set ab initio method.

The accuracy of the optimized geometries obtained by MNDO calculations for the chloroethylene oxides cannot be judged, as there is no available experimental data. However, calculations on these molecules have been done using the ab initio method (Table III) [61]. Comparing results from the two methods, the average discrepancy in C—C bond length is 0.03 Å and about 3° for the bond angles. As with the parent compounds, there are also differences in the dipole moments calculated by the two methods.

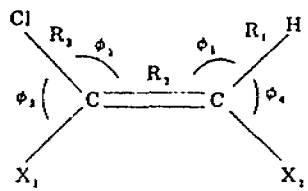
Experimental structural data for the product aldehydes are limited. As seen in Table IV, MNDO's optimized structures are in good agreement with available data. As with the parent compounds, the largest discrepancies are in the bond lengths and angles of the carbon-chlorine bond. MNDO consistently overestimates these bond lengths by about 0.03 Å and maximum bond angle errors are about 3°.

The accuracy of heats of formation calculated for the parent chloroethylenes and carbonyl products is shown in Table V. While the general agreement with experiment is good, the error increases with degree of chlorination and, for the fully chlorinated trichloroacetylchloride, is substantial.

No experimental data exist for the heat of formation of the epoxides. However, a comparison of the heats of reaction by MNDO for the transformation of the parent to the epoxide and those done by the STO-3G methods yields an interesting result (Table V(B)). MNDO calculations give a much greater dependence of heats of formation on degree of halogen substitution than those done by STO-3G. Since MNDO is parameterized for heats of formation, it is likely that it yields a more accurate relative result than the STO-3G method.

TABLE II

CALCULATED GEOMETRIES AND DIPOLE MOMENTS OF PARENT COMPOUNDS AND COMPARISON WITH EXPERIMENT

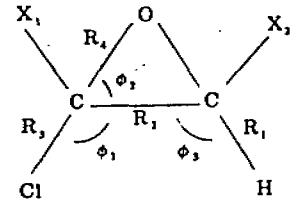


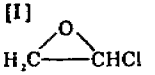
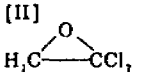
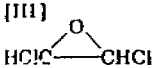
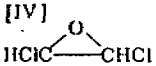
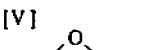
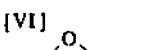
		R_1	R_2	R_3	ϕ_1	ϕ_2	ϕ_3	ϕ_4	μ
Vinyl chloride							$X_1 = H$		
H_2C—CHCl	MNDO	1.09	1.33	1.76	124°	123°	111°	—	1.71
	STO-3G[c]	1.08	1.31	1.77	122°	123°	116°	—	2.22
	Exp[a]	1.10	1.34	1.73	121°	122.5°		—	1.44[e]
Vinylidene chloride							$X_1 = Cl$	$X_2 = H$	
Cl_2C—CH_2	MNDO	1.09	1.34	1.74	—	—	114°	113.2	1.86
	STO-3G[c]	1.08	1.31	1.77	—	—	118°	115	2.34
	Exp[b]	—	1.324	1.707	—	—	113.6°	—	1.25[d]
1,2-Dichloroethylene (*cis*)									
HClC—CHCl	MNDO	1.09	1.34	—	121°	125.5°	—	—	2.13
	STO-3G[c]	1.08	1.31	—	123°	125°	—	—	3.02
	Exp[b]	—	1.34	—	—	—	—	—	1.80[d]
1,2-Dichloroethylene (*trans*)									
HClC—CHCl	MNDO	1.09	1.34	1.75	127°	121°	—	—	0.009
	STO-3G[c]	1.08	1.31	1.77	124°	124°	—	—	0
	Exp[b]	—	1.34	1.72	—	123°	—	—	0[d]
Trichloroethylene							$X_1 = Cl$		
HClC—CCl_2	MNDO	1.09	1.34	1.74	—	124°	115°	—	1.13
	STP-3G[c]	1.08	1.32	1.76	—	124°	116°	—	1.61
	Exp	—	1.36	1.71	—	124°	117—123.75°	—	0.86[d]
Tetrachloroethylene							$X_1 = Cl$		
Cl_2C—CCl_2	MNDO	—	1.36	1.74	—	—	115°	—	0.001
	STO-3G[c]	—	1.33	1.77	—	—	118°	—	0
	Exp[b]	—	1.33	1.73	—	—	—	—	0[d]

ethylene	MNDO	1.09	1.34	1.74	—	124°	X_1 = Cl		—	1.13
HClC—CCl_2	STP-3G[c]	1.08	1.32	1.76	—	124°	116°		—	1.61
	Exp	—	1.36	1.71	—	124°	117—123.75°		—	0.85[d]
Tetrachloro-ethylene	MNDO	—	1.35	1.74	—	—	X_1 = Cl 115°		—	0.001
Cl_2C—CCl_2	STO-3G[c]	—	1.33	1.77	—	—	112.8°		—	0
	Exp[b]	—	1.32[d]	1.72	—	—	118°		—	0[d]

TABLE III

COMPARISON OF CALCULATED GEOM... STO-3G

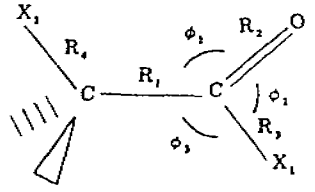


		R_1	R_2	R_3	R_4	ϕ_1	ϕ_2	ϕ_3	ϕ_4	μ
[I]									X_1 = H	
	MNDO	1.10	1.52	1.78	1.42	121°	57°	121°	114°	2.386
	STO-3G[a]	1.09	1.48	1.80	1.42	120°	58°	119°	111°	2.448
[II]									X_1 = Cl	
	MNDO	1.10	1.52	1.77	1.43	122°	56°	112°	116°	2.362
	STO-3G[a]	1.08	1.49	1.79	1.41	120°	60°	119°	113°	2.476
[III]									X_1 = H	
	MNDO	1.10	1.52	1.77	1.41	123°		123°	109°	2.709
(*cis*)	STO-3G[a]	1.09	1.49	1.79	1.43	122°		120°	112°	2.167
[IV]									X_1 = H	
	MNDO	1.10	1.52	1.79	1.41	121°	57°		114°	1.049
(*trans*)	STO-3G[a]	1.09	1.49	1.79	1.42	121°	58°		112°	0.458
[V]									X_1 = Cl	
	MNDO		1.53	1.77	1.41	120°	56°	—	147°	1.597
	STO-3G[a]		1.50	1.79	1.41	119°	59°	—	112°	1.747
[VI]									X_1 = Cl	
	MNDO	—	1.54	1.76	1.40	122°	57°	—	110°	0.664
	STO-3G[a]	—	1.51	1.79	1.43	121°	—	—	113°	0.113

[a] Ref. 60.

TABLE IV

MNDO CALCULATED GEOMETRIES OF CHLOROALDEHYDE AND ACYLCHLORIDE PRODUCTS AND COMPARISON WITH EXPERIMENT



		R_1	R_2	R_3	R_4	ϕ_1	ϕ_2
$H_3C-\overset{O}{\overset{\|}{C}}-H$				$X_1 = H$	$X_2 = H$		
	MNDO	—	1.22	1.11	1.11	125°	—
	exp[a]	—	1.22 ± 0.02	1	1.09	121°	—
[I]							
$H_3C-\overset{O}{\overset{\|}{C}}-Cl$				$X_1 = Cl$			
	MNDO	1.51	1.21	1.80	1.11	—	119°
	exp[a]	1.50	1.22 ± 0.04	1.77	1.09	—	
[II]							
$Cl_2HC-\overset{O}{\overset{\|}{C}}-Cl$				$X_1 = Cl$	$X_2 = Cl$		
	MNDO	1.53	1.205	1.79	1.79	112.4°	119.57°
	exp[a]	1.52 ± 0.04	1.21 ± 0.04	1.74 ± 0.03	1.76 ± 0.03	113 ± 3°	122 ± 3°
[III] [IV]							
$Cl_2C-\overset{O}{\overset{\|}{C}}-H$					$X_2 = Cl$		
	MNDO	1.534	1.206	—	1.79	—	—
	exp[a]	1.52 ± 0.02	1.15 ± 0.02	—	1.76 ± 0.02	—	—

[a] Ref. 60.

Cl_2CCH (O)	MNDO	1.534	1.206	X_1 = Cl 1.79	—	—
	exp[a]	1.52 ± 0.02	1.15 ± 0.02	1.76 ± 0.02	—	—

[a] Ref. 60.

TABLE V

COMPARISON OF MNDO CALCULATED HEATS OF FORMATION OF THE CHLOROETHYLENES, EPOXIDES, ALDEHYDES AND ACYLCHLORIDES WITH EXPERIMENTAL DATA AND WITH STO-3G AB INITIO CALCULATIONS

(A)			(B)			(C)		
Parent compound	ΔH_f (MNDO) (kcal/mol)	ΔH_f(exp) (kcal/mol)	Corresponding epoxide	$\Delta(\Delta H)$[a] STO-3G[a]	$\Delta(\Delta H)$[a] (MNDO)	Carbonyl compound	ΔH_f (MNDO) (kcal/mol)	ΔH_f (exp) (kcal/mol)
H_2C—$CHCl$	4.81	8.1[c]	H_2C(O)$CHCl$	0	0	H_2ClCCH (O)	49.67	−61.3[e]
H_2C—CCl_2	−0.16	0.61 ± 0.36[d]	H_2C(O)CCl_2	2.92	1.00	H_2CCCl (O)	−56.13	
HClC—CClH (*cis*)	−2.82	1.0 ± 2.1[d]	(*cis*) HClC(O)CHCl	2.45	0.00	H_2ClCCl (O)	−60.33	−68.9[e]
HClC—CClH (*trans*)	−3.90	1.2 ± 2.1[d]	(*trans*) HClC(O)CHCl	1.95	1.00	HCl_2CCH (O)	−52.81	−51.1[e]
Cl_2C—$CHCl$	−6.60	—	Cl_2C(O)$CHCl$	4.89	1.00	HCl_2CCCl (O)	−60.19	
Cl_2C—CCl_2	−8.21	−2.7 ± 2.0[d]	Cl_2C(O)CCl_2	7.18	1.00	Cl_2CCH (O)	−43.83	−51.1[e]
						Cl_2CCCl (O)	−55.45	−89.3[d]

[a] $\Delta(\Delta H) = (\Delta H_{f_{epoxide}} - \Delta H_{f_{parent}})$ relative to values calculated for vinyl chloride.
[b] Ref. 60.
[c] S.W. Benson, F.R. Cruickshank, D.M. Golden, G.R. Hargen, H.E. O'Neal, A.S. Rogers, R. Shaw and R. Walsh, Chem. Rev., 69 (1909) 279.
[d] J.D. Cox and G. Pilcher, Thermochemistry of Organic and Organometallic Compounds, Academic Press, London, 1970.
[e] Selected Values of Chemical Thermodynamic Properties, National Bureau of Standards, Washington, DC.

TABLE VI

CALCULATED HEATS OF FORMATION OF REACTANTS, PRODUCTS AND INTERMEDIATES IN PROPOSED TRANSFORMATION OF CHLOROETHYLENES TO PUTATIVE ULTIMATE CARCINOGENS: CHLOROALDEHYDES AND ACYLCHLORIDES (PATHWAY I)

Parent	$\Delta(\Delta H_f)$ Triplet - Parent	Triplet Biradical	$\Delta(\Delta H_f)$ Isomerization	Isomerized[a] Triplet Biradical	$\Delta(\Delta H_f)$ Product - Biradical	Final Product	$\Delta(\Delta H_f)$ Product - Parent
(I) ΔH_f = 4.81	2.88	ΔH_f = 7.69	-12.74	ΔH_f = 5.05	-44.62	ΔH_f = -48.87	-54.48
	3.70	ΔH_f = 8.51	-13.56 / -10.40	ΔH_f = -1.89	-54.24	ΔH_f = -56.13	-60.94
(II) ΔH_f = -0.16	8.14	ΔH_f = 7.98	-16.90	ΔH_f = -8.92	51.41	ΔH_f = -60.33	-60.17
	1.11	ΔH_f = 0.95	-8.41	ΔH_f = -7.46	-45.35	ΔH_f = -52.81	-52.65
(III) ΔH_f = -3.90	3.14	ΔH_f = -0.76	-8.16	ΔH_f = -8.92	-51.41	ΔH_f = -60.33	-56.43
			-6.70	ΔH_f = -7.46	-45.35	ΔH_f = -52.81	-48.91
(IV) ΔH_f = -2.82	2.06	ΔH_f = -0.76	-8.16	ΔH_f = -8.92	-51.41	ΔH_f = -60.33	-57.51
			-6.70	ΔH_f = -7.46	-45.35	ΔH_f = -52.81	-50.00
(V) ΔH_f = -6.80	5.39	ΔH_f = -5.10	-5.29	ΔH_f = -4.96	-44.87	ΔH_f = -49.83	-43.23
	1.50	ΔH_f = -0.21	-10.18 / -9.18	ΔH_f = -10.39	-50.57	ΔH_f = -60.91	-54.31
(VI) ΔH_f = -8.21	6.53	ΔH_f = -1.68	-3.46	ΔH_f = -5.14	-50.31	ΔH_f = -55.45	-47.24

[a] Isomerization resulting from a 1,2 $H^{\cdot}$ or $Cl^{\cdot}$ atom shift.
*Observed product found in vivo or in vitro using rat liver.
† Predicted pathway.

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TABLE VII

CALCULATED HEATS OF FORMATION OF REACTANTS, PRODUCTS AND INTERMEDIATES IN PROPOSED TRANSFORMATION OF CHLOROETHYLENES TO PUTATIVE ULTIMATE CARCINOGENS: EPOXIDES (PATHWAY II)

Parent	$\Delta(\Delta H_f)$ Triplet - Parent	Triplet Biradical	$\Delta(\Delta H_f)$ Singlet - Triplet	Singlet Biradical	$\Delta(\Delta H_f)$ Ring Closing Epoxide - Singlet	Epoxide	$\Delta(\Delta H_f)$ Epoxide - Parent
(I) ΔH_f = 4.81	2.88	ΔH_f = 7.69	0.41	ΔH_f = 8.10	-31.72	ΔH_f = -73.62	-28.43
	2.70	ΔH_f = 8.51	1.46	ΔH_f = 8.97	-33.59		
(II) ΔH_f = -0.16	8.14	ΔH_f = 7.98	0.22	ΔH_f = 8.75	-69.08	ΔH_f = -25.67	-25.†
	1.10	ΔH_f = 0.95	0.77	ΔH_f = 1.27	-54.08		
(III) ΔH_f = 3.90	2.06	ΔH_f = -0.76	1.11	ΔH_f = 0.35	-30.73	ΔH_f = -30.38	-26.48
(IV) ΔH_f = -2.82	3.14	ΔH_f = -0.78	1.11	ΔH_f = 0.35	-30.73	ΔH_f = -30.38	-27.56
(V) ΔH_f = -6.60	1.48	ΔH_f = -0.21	1.45	ΔH_f = 1.24	-31.38	ΔH_f = -30.14	-23.54
		ΔH_f = -5.10	1.0	ΔH_f = -4.10	-26.04		
(VI) ΔH_f = -8.21	6.53	ΔH_f = -1.68	2.11	ΔH_f = 0.43	-29.83	ΔH_f = -29.46	-21.25

[a] Not fully optimized.
*Observed product found in vivo or in vitro using rat livers.
†Predicted pathway.

TABLE VIII

CALCULATED HEATS OF FORMATION OF SPECIES INVOLVED IN PROPOSED FORMATION OF CARBONYL PRODUCTS FROM EPOXIDES OF CHLOROETHYLENES BY PATHWAY III

Epoxide	$\Delta(\Delta H_f)$ Protonated Epoxide - Epoxide	Protonated Epoxide	$\Delta(\Delta H_f)$ Ring Opening	Carbocation	$\Delta(\Delta H_f)$ Isomerization	Isomerized[a] Carbocation	$\Delta(\Delta H_f)$ Aldehyde - Carbocation	Final Product	$\Delta(\Delta H_f)$ Final Product - Epoxide
(I) ΔH_f = -23.62	197.67	ΔH_f = 174.06	-3.00	ΔH_f = 171.05	-30.10	ΔH_f = 140.95	-190.62	ΔH_f = -49.67	-26.05
			+5.09	ΔH_f = 179.14	-40.81	ΔH_f = 138.33	-184.46	ΔH_f = -56.33	-32.61
(II) ΔH_f = -25.67	204.15	ΔH_f = 178.48	4.50	ΔH_f = 182.98	-42.85	ΔH_f = 140.13	-200.46	ΔH_f = -60.33	-34.66
			-10.15	ΔH_f = 168.33	-22.77	ΔH_f = 145.56	-198.37	ΔH_f = -62.81	-37.15
(III) (IV) ΔH_f = -30.38	206.08	ΔH_f = 175.70	-3.62	ΔH_f = 172.08	-31.95	ΔH_f = 140.13	-200.46	ΔH_f = -60.33	-29.95
					-25.52	ΔH_f = 145.56	-188.37	ΔH_f = -62.81	-22.43
(V) ΔH_f = -30.14	210.82	ΔH_f = 180.68	-4.35	ΔH_f = 176.33	-29.34	ΔH_f = 146.99	-205.18	ΔH_f = -60.19	-30.08
			-11.40	ΔH_f = 169.25	-16.60	ΔH_f = 152.64	-201.47	ΔH_f = -48.83	-18.69
(VI) ΔH_f = -29.46	215.56	ΔH_f = 186.10	-12.35	ΔH_f = 173.75	-19.69	ΔH_f = 154.08	-209.51	ΔH_f = -55.45	-25.99

[a] Isomerization resulting from a 1,2 H^- or Cl^- ion shift.
[b] Not fully optimized.
[c] Product observed in non enzymatic transformation of epoxide.
* Final product observed in vivo or in vitro using perfused rat livers.
† Predicted pathway.

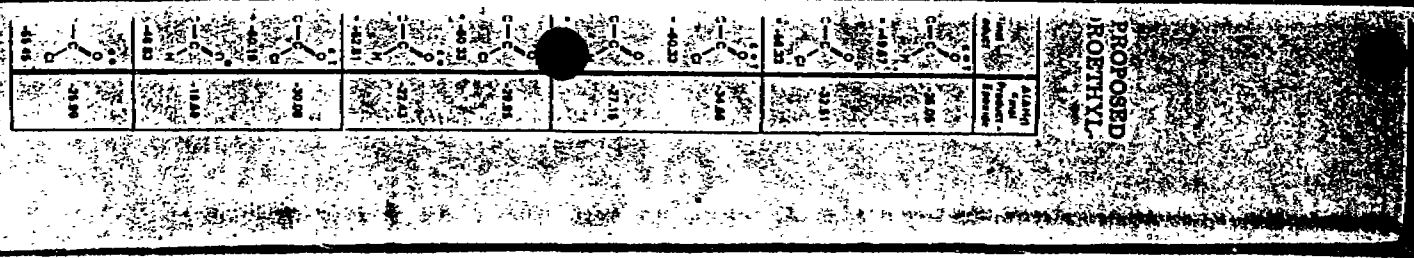

TABLE IX

SPIN DENSITIES ON CARBON AND OXYGEN ATOMS IN POSTULATED BIRADICAL INTERMEDIATES IN FORMATION OF ALDEHYDES OR ACYLCHLORIDE ULTIMATE CARCINOGENS

		Compound	q_{C_α}	q_{C_o}	q_O
I	Radical	$HClC^{\bullet}-CH_2O^{\bullet}$	+1.09	+0.16	+0.96
	H-shift	$ClH_2C-C^{\bullet}HO^{\bullet}$	+0.02/−0.02	+0.79	+1.10
	Radical	$H_2C^{\bullet}-CHClO^{\bullet}$	+1.20	−0.14	+0.96
	H-shift	$H_2C^{\bullet}-C^{\bullet}ClO^{\bullet}$	+0.03	+0.82	+1.08
	Cl-shift	$ClH_2C-C^{\bullet}HO^{\bullet}$	+0.02/−0.02	+0.79	+1.10
II	Radical	$Cl_2C^{\bullet}-CH_2O^{\bullet}$	+1.04	+0.15	+0.96
	H-shift	$HCl_2C-C^{\bullet}HO^{\bullet}$	+0.02/−0.01	+0.78	+1.11
	Radical	$H_2C^{\bullet}-CCl_2O^{\bullet}$	+1.19	+0.02	+0.96
	Cl-shift	$ClH_2C-C^{\bullet}ClO^{\bullet}$	+0.00/−0.00	+0.77	1.09
III, IV	Radical	$HClC^{\bullet}-CHClO^{\bullet}$	+1.01	+0.16	+0.95
	H-shift	$H_2ClC-C^{\bullet}ClO^{\bullet}$	+0.00/−0.00	+0.77	+1.09
	Cl-shift	$HCl_2C-C^{\bullet}HO^{\bullet}$	+0.02/−0.01	+0.78	+1.11
VI	Radical	$HClC^{\bullet}-CCl_2O^{\bullet}$	+1.12	−0.17	−0.95
	Cl-shift	$HCl_2C-C^{\bullet}ClO^{\bullet}$		+0.77/−0.01	+1.11
	Radical	$Cl_2C^{\bullet}-CHClO^{\bullet}$	+1.06	+0.16	+0.95
	Cl-shift	$Cl_2C-C^{\bullet}HO^{\bullet}$	+0.02/−0.01	+0.78	+1.12
	H-shift	$HCl_2C-C^{\bullet}ClO^{\bullet}$		+0.77/−0.01	+1.11
	Radical	$Cl_2C^{\bullet}-CCl_2O^{\bullet}$	+0.91	−0.16	+0.97
	Cl-shift	$Cl_3C-C^{\bullet}ClO^{\bullet}$	+0.03	+0.77/−0.01	+1.11

TABLE X

CALCULATED ELECTROPHILIC PROPERTIES OF ULTIMATE CARCINOGENS: ACYLCHLORIDES AND ALDEHYDES

Parent compound	Postulated ultimate carcinogens	q_{C_o}[f]	q_{C_α}[g]	E_{π^*LUMO}[h] (ev)	LCAO(C_π)[i]	LCAO($C_{\alpha\pi}$)[i]
[I][a]	$ClCH_2\overset{O}{\overset{\|}{C}}H$[b,1]	+0.2435	+0.0577	−0.1520	+0.559	+0.419
	$CH_3\overset{O}{\overset{\|}{C}}Cl$[1]	+0.3144	+0.0162	−0.0730	+0.778	+0.069
[II]	$ClCH_2\overset{O}{\overset{\|}{C}}Cl$[c,1]	+0.3021	+0.1160	−0.6700	+0.511	+0.547
[III], [IV]	$Cl_2CH\overset{O}{\overset{\|}{C}}H$[d]	+0.2527	+0.0937	−0.8381	+0.536	+0.357
[V]	$Cl_2\overset{O}{\overset{\|}{C}}H$[e]	+0.2590	+0.1592	−1.033	+0.439	+0.599
	$CHCl_2\overset{O}{\overset{\|}{C}}Cl$	+0.2985	+0.1518	−1.209	+0.557	+0.490
[VI]	$Cl_2\overset{O}{\overset{\|}{C}}Cl$[e]	+0.2952	+0.1908	−1.496	+0.499	+0.539

[a] Numerals in brackets refer to parent compound as labeled in Table VI from which carbonyl compound listed was formed.
[b] Observed product of parent compound I.
[c] Observed product of parent compound II.
[d] Observed product of parent compounds III and IV.
[e] Observed product of parent compounds V and VI.
[f] $q_{C=O}$: net charge on carbonyl carbon calculated by Muliken population analysis.
[g] q_α: net charge on α carbon calculated.
[h] E_{LUMO}: calculated energy of lowest unoccupied molecular orbital.
[i] E_{LUMO} values calculated by ab initio method using STO-3G basis set for these compounds are +0.110 ev, +0.122 ev and +0.0755 ev, respectively.

Thermodynamic data for the three proposed pathways (Figs. 3 and 4) to formation of chloracetaldehydes, acylchlorides and epoxides are summarized in Tables VI–VIII, respectively. We see from Tables VI and VII that the proposed first step, addition of a triplet oxygen atom to the parent compound, can yield two different radical intermediates corresponding to addition of an oxygen atom to each of two inequivalent carbon atoms. As shown in Table VI, Pathway I to carbonyl products is

TABLE XI

CALCULATED ELECTROPHILIC PROPERTIES OF CARBOCATIONS ALTERNATIVE POSSIBLE ACTIVE FORMS OF CHLOROETHYLENES

Parent	Protonated Epoxide	Carbocation	q_C^+	Protonated Epoxide E_{LUMO} (ev)	Carbocation E_{LUMO} (ev)
(I)	ΔH_f = 174.05	ΔH_f = 171.66	+.409	−5.62	−8.67
		ΔH_f = 176.14	+.536		−9.04
(II)	ΔH_f = 179.48	ΔH_f = 182.86	+.561	−6.09	−9.35
		ΔH_f = 168.33	(+.330)		−8.71
(III, IV)	ΔH_f = 175.70	ΔH_f = 172.08	+.412	−5.92	−9.05
(V)	ΔH_f = 180.66	ΔH_f = 178.33	+.417	−6.25	−9.27
		ΔH_f = 187.25	+.326		−9.03
(VII)	ΔH_f = 188.10	ΔH_f = 177.75	+.329	−6.43	−9.23

[a] Observed product of parent compounds V and VI.
[b] $q_{C=O}$: net charge on carbonyl carbon calculated by Mulliken population analysis.
[c] q_α: net charge on α carbon calculated.
[d] E_{LUMO}: calculated energy of lowest unoccupied molecular orbital.
[e] E_{LUMO} values calculated by ab initio method using STO-3G basis set for these compounds are +0.110 ev, +0.122 ev and +0.0755 ev, respectively.

further complicated by the fact that isomerization of these initial triplet radicals by H or Cl atom 1, 2 shifts can also lead to multiple species.

Table IX gives the calculated spin densities on all biradical intermediates on the formation of aldehyde or acylchloride intermediates. We see from Table IX that in the initial biradical formed, one unpaired electron is on the alpha carbon atoms and one on the oxygen, with some delocalization on the carbonyl carbon. Isomerization of this triplet biradicals by 1, 2 H or Cl atom shifts, leads to a corresponding shift in unpaired electron density from the alpha carbon, which is tetrahedral in the isomerized radical to the carbonyl carbon prior to reforming the C—O double bond.

Table X gives the calculated properties of the carbonyl compounds chosen as indicators of their relative electrophilicity, i.e., net charge on the two carbon atoms, the energy of the lowest unoccupied π^* molecular orbital and the extent of participation of the two carbon atoms in this orbital. As shown in Table X, this π orbital has significant participation of both carbon atoms. In would serve as the initial electron accepting orbital in covalent bond formation with tissue nucleophiles. The energy of this orbital is an indication of the ease of electron transfer to the active form of the carcinogen in covalent bond formation with DNA bases.

The MNDO method tends to overestimate the stability of the low-lying empty orbitals. As with all other properties, however, only the relative value of E_{LUMO} obtained by MNDO for the series of related carbonyl compounds is used.

In order to verify whether the same relative behavior occurs using a more rigorous method of calculation, E_{π^*LUMO} was calculated for three representative aldehydes: monochloroacetylchloride, monochloroacetaldehyde and acylchloride, using an ab initio method with an STO-3G basis set [61]. The values obtained are given in the footnote to Table X. We see that, while the absolute values obtained are different for each analog, the order of increasing electron affinity is preserved.

Table XI summarizes similar calculated electrophilic properties of carbocations resulting from ring opening of protonated epoxides.

DISCUSSION

Electrophilicity of putative ultimate carcinogens and correlation with carcinogenic potency

Chloroaldehydes and acylchlorides have been postulated as a plausible active form of chloroethylenes. However, it is also possible that epoxide intermediates can form the observed DNA adducts, provided they are protected from the facile rearrangement to carbonyl products which both our calculations and experimental observations indicate occur.

If acylchlorides and aldehydes are the ultimate carcinogens, the type of adducts observed (Fig. 2) require that they act as electrophiles. Our results, however, indicate no correlation between calculated indicators of electrophilicity of the putative ultimate carcinogens and the tentative rank order of

carcinogenic activity (Table XII). This lack of correlation holds for all possible chloroaldehydes or acylchlorides of the parent compound, whether or not they or their oxidized and reduced forms have been identified as metabolites.

These results are in striking contrast to those we obtained in similar studies of the saturated chloroethanes (Loew and Rebagliati, unpublished). Though presumed to act via the same ultimate carcinogens, the chloroethanes undergo a different transformation to the reactive species than that of the chloroethylenes. The relative electrophilicities of the chloroacetaldehydes and acylchlorides are excellent indicators of their work order of carcinogenic activity for this class of compounds. The results obtained here and the differences between them and those for the saturated chloroethanes have three major implications:

(1) For the unsaturated chloroethylenes, the rather complex transforma-

TABLE XII

CORRELATION OF CALCULATED PROPERTIES WITH RELATIVE CARCINOGENICITY OF PARENT CHLOROETHYLENES ASSUMING CARBONYL PRODUCTS AS ULTIMATE CARCINOGENS

Parent compounds	Rank order carcinogenicity parent	Postulated ultimate carcinogen	q_{C_α}	E_{LUMO} (ev)	$\Delta(\Delta H_{iR})$ (kcal/mol)	$\Delta(\Delta H_{iep})$ (kcal/mol)
$Cl_2C{=}CHCl$	1	$ClCH_2\overset{O}{\overset{\|}{C}}H$[a,b]	+0.058	−0.152	0	0
$H_2C{=}CCl_2$	1	$H_2ClC\overset{O}{\overset{\|}{C}}Cl$[a,b]	+0.116	−0.670	−4.2	−4.7
HClC=CClH	2	$HCl_2C\overset{O}{\overset{\|}{C}}{-}H$[b]	+0.09	−0.838	6.0	11.7
		$H_2ClC\overset{O}{\overset{\|}{C}}Cl$[a]	+0.116	−0.670	4.6	6.2
$Cl_2C{=}CCl_2$	3	$Cl_3C\overset{O}{\overset{\|}{C}}Cl$[a,b]	+0.191	−1.496	9.3	18.7
HClC=CCl_2	4	$Cl_2C\overset{O}{\overset{\|}{C}}H$[c]	+0.159	−1.033	12.9	21.6
		$Cl_2HC\overset{O}{\overset{\|}{C}}Cl$[a,d]	(0.152)	(−1.209)	(7.45)	(15.4)

[a] Predicted products.
[b] Observed products.
[c] Product observed from parent compound in vivo.
[d] Product observed from epoxide in vitro.

TABLE XIII

CORRELATION OF CALCULATED PROPERTIES WITH RELATIVE CARCINOGENICITY OF PARENT CHLOROETHYLENES ASSUMING EPOXIDE CARBOCATION AS ULTIMATE CARCINOGEN

Parent compounds	Rank order carcinogenicity parent	Postulated ultimate carcinogen	q_{C_α}	$\Delta(\Delta H_{ep})$
$H_2C{=}CHCl$	1	$H_2C^+{-}CHClOH$	+0.536	0
$H_2C{=}CCl_2$	2	$H_2C^+{-}CCl_2OH$	+0.561	2.3
$HClC{=}CHCl$	?	$HClC^+{-}CHClOH$	0.412	2.5
$Cl_2C{=}CCl_2$	3	$Cl_2C^+{-}CCl_2OH$	0.329	7.2
$CHC{=}CCl_2$	4	$Cl_2C^+{-}CHClOH$	0.326	4.7

tion to the ultimate carcinogens is an important discriminating factor in determining their relative carcinogenic activity, compared to the electrophilicity of the resultant species.

(2) For the unsaturated chloroethylenes, there could be competing detoxification pathways involving the electrophilicity of the ultimate carcinogens which impede their interactions with DNA.

(3) If carbocations formed from epoxides by protonation and ring opening are the ultimate carcinogens, their electrophilicity could be relevant. As shown in Table XI, the E_{LUMO}-values for these cations are relatively constant for the series of compounds ($E_{LUMO} = -9.04$ to -9.23 ev). They do not correlate with observed relative carcinogenic activity of the parent compounds. However, as shown in Table XIII, there is some correlation of relative carcinogenic activity with the calculated net charge on the cationic carbon of each epoxide carbocation that could be involved in Type 2 adduct formation.

Correlations of thermodynamic properties with the extent of metabolism and carcinogenicity

The remaining results obtained in this study, i.e., calculated stabilities of intermediates and products, can be used to investigate the hypothesis that the extent of transformations of parent chloroethylenes to the putative ultimate carcinogens determines their relative carcinogenic potency and to obtain insights into the mechanisms of these transformations. To this end, comparison of predicted and observed relative metabolic behavior of the six chloroethylenes should help decide which, if any, of the proposed pathways is relevant to formation of the active carcinogen. Examination of all calculated thermodynamic quantities for their extent of correlation with

rank order of carcinogenicity should help determine if extent of metabolism is a discriminating factor in relative carcinogenic activity.

As discussed above, we have proposed that transformation of chloroethylenes to carbonyl products can proceed by three different pathways: triplet biradical isomerization (Pathway I), a triplet/singlet crossing (Pathway II), or via an epoxide intermediate (Pathway III) (Fig. 3). Epoxide formation is also possible by Pathway II.

Support for this mechanism comes from the result that calculated energy differences between triplet and singlet biradicals shown in Table VII are small. The optimized geometries for the two states are also close. For example, the largest discrepancy in bond lengths between the triplet and singlet states of *HClC—CHClO* is *0.004 Å for the C—O bond* and in bond angles is 1°. Thus, intersystem crossing could occur within the energetic and geometrical 'boundaries' of a molecule's normal vibrational modes.

All relevant thermodynamic data are presented in Tables VI—VIII. The use of the calculated results to interpret experimental metabolic studies is complicated by the fact that, while there is only one epoxide intermediate possible for each parent compound, multiple radicals and isomerized radicals and carbocations are predicted from all but the perchlorinated parent compound, leading to two possible carbonyl products. The favored route to product formation by each pathway is shown in each table. In enzymatic reactions it is difficult to determine which of the many complex steps leading to carbonyl products determines the extent of metabolism and product specificity. Hence the intermediates calculated to be most stable do not always correspond to these products identified thus far in in vivo metabolism. Other factors which could introduce uncertainties in the usefulness of our calculated quantities as indicators of both metabolic and carcinogenic behavior are: (1) we have considered only electronic components of enzymatic reactions, neglecting steric effects which cannot be modeled without detailed knowledge of the active site of the enzymes; (2) we are using thermodynamic, rather than kinetic, criteria for extent and specificity of formation of metabolic products, i.e., calculation of heats of formation of intermediates rather than activation energies of transition states; (3) errors in calculated heats of formations. Given these uncertainties, however, our results do give some insights into mechanism of transformation.

Comparing our predicted products with those observed from metabolic studies [16,24,26,27,36—42] involving either whole animals, perforated liver or liver microsomes, we have predicted the major metabolites observed for vinyl chloride and vinylidene chloride, but not for trichloroethylene. However, even for vinylidene chloride, most of the lowest energy intermediates leading to the observed product result from the initial triplet radical with a calculated higher energy, indicating this first step is not rate determining.

Our results for *cis*- and *trans*-1,2-dichloroethylene lead to prediction of monochloroacetyichloride as a major product. While preliminary metabolic

studies using perfused liver failed to detect this metabolite [24], more work is needed to clarify whether it, as well as dichloroacetic acid, is present.

Our results for trichloroethylene lead to the prediction of dichloroacetylchloride and its products as metabolites, whereas the other possible metabolite, trichloroacetaldehyde (chloral), has been observed as well as trichloroacetic acid and chloral hydrate, $Cl_3CCH(OH)_2$ [41]. Our predicted metabolite has, however, been found in studies of non-enzymatic transformation of the epoxide, a result in agreement with the energetics of Pathway III.

Pathway III describes a possible non-enzymatic transformation of epoxides to aldehydes. Favorable energetics are obtained for each step in the proposed pathway, and, in particular, isomerized carbocations formed by both chlorine and hydrogen migration are more stable than the original carbocation, indicating Pathway III is plausible. Consistent with these results, synthetic epoxides were found to rearrange thermally to form acylchlorides and di- and monochloroacetaldehyde [24].

The products detected all correspond to chlorine migration which is calculated to be competitive with hydrogen migration for all compounds except *cis*- and *trans*-1,2-dichloroethylene. For these two compounds, our results favor hydrogen migration leading to monochloroacetic acid as a major product even by non-enzymatic transformation of the epoxide. In general, our results indicate non-enzymatic isomerization which appears to be the determining step in carbonyl product selectivity.

Of all the thermodynamic quantities calculated for these complex transformations of chloroethylenes, only the four given in Table XIV correlate with their rank order of metabolism. Given in Table XIV are: (1) the relative enthalpies of isomerization of the initial triplet biradicals $\Delta(\Delta H_{iR})$, leading directly to carbonyl products; (2) the differences in enthalpy between the triplet radical and singlet biradical $\Delta(\Delta H_{ST})$; (3) the stabilities of epoxide intermediates formed from the singlet biradical relative to each parent compound $[\Delta(\Delta H_{ep})]$; (4) the relative enthalpies of isomerization of these epoxides to carbonyl products $[\Delta(\Delta H_{iep})]$. Consistently, as discussed above, the energetics of these steps also appear to be most relevant in determining product specificities.

Having identified four thermodynamic quantities which correlate with the extent of metabolism of parent compounds, the question arises whether any of them also contribute to their relative carcinogenic activity. If carbonyl products are assumed to be the ultimate carcinogens, as shown in Table XII, their relative isomerization enthalpies both with and without epoxide intermediates are good indicators of rank order of carcinogenic potency. These correlations imply both pathways to the carbonyl products can contribute to observed carcinogenic activity of the parent compounds.

Interestingly, if we assume dichloroacetylchloride, our predicted metabolite and that found in vitro, to be the ultimate carcinogen of trichloroethylene, we predict it to be more carcinogenic than tetrachloroethylene; whereas with the observed product, it is predicted to be less carcinogenic.

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TABLE XIV

CORRELATION OF CALCULATED PROPERTIES WITH EXTENT OF METABOLISM OF CHLOROETHYLENES

Compounds	Product	Rank of metabolism	$\Delta(\Delta H_{(R}$[c]	$\Delta(\Delta H_{ep})$[d]	$\Delta(\Delta H_{iep})$[e]	$\Delta(\Delta H_{ST})$[f]
$H_2C{=}CHCl$	$H_2ClC{-}\overset{O}{\overset{\|}{C}}H$[a,b]	1	0	0	0	0
$H_2C{=}CCl_2$	$H_2ClC{-}\overset{O}{\overset{\|}{C}}Cl$[a,b]	1	−4.16	2.29	−4.66	−1.0
$HClC{=}CClH$ (*cis*, *trans*)	$HCl_2{-}\overset{O}{\overset{\|}{C}}{-}H$[b]	2	6.04	2.45 (*cis*) (1.95) (*trans*)	11.67	0.70
	$H_2Cl{-}\overset{O}{\overset{\|}{C}}Cl$[a]		4.57	2.45 (*trans*) (1.95) (*cis*)	6.24	0.70
$Cl_2C{=}CHCl$	$Cl_2C{-}\overset{O}{\overset{\|}{C}}H$[b]	3	12.88	4.73	21.59	0.56
	$Cl_2HC{-}\overset{O}{\overset{\|}{C}}{-}Cl$[a]	3	7.45	4.73	15.43	0.57
$Cl_2C{=}CCl_2$	$Cl_2C{-}\overset{O}{\overset{\|}{C}}Cl$[a,b]	4	9.28	7.18	18.69	1.70

[a] Predicted metabolite.
[b] Observed metabolite.
[c] $\Delta(\Delta H_{(R})$ = triplet biradical isomerization enthalpies.
[d] $\Delta(\Delta H_{ep})$ = stability of epoxide, i.e.: $\Delta H_{f(\text{epoxide})} - \Delta H_{f(\text{parent})}$.
[e] $\Delta(\Delta H_{iep})$ = isomerization energy of carbocations of epoxides.
[f] $\Delta(\Delta H_f)_{ST}$ = difference in enthalpy between triplet radical and singlet biradical. All quantities relative to most favorable enthalpy differences.

These results suggest that the lower activity found for trichloroethylene is due to competing formation of other products, perhaps by hydrolysis of the epoxide. Determination of the effect of epoxide hydrase on metabolic distribution and carcinogenic activity could help resolve this point.

Experiments with epoxide hydrase could also help resolve the question of whether epoxides themselves or their carbocations also act as ultimate carcinogens. As indicated in Table XIII, two calculated properties, the net charge on the epoxide carbocation and the stability of the epoxide relative to the parent compound, also show some correlation with carcinogenic activity and could implicate these species as ultimate carcinogens.

For the chloroethylenes, then, three possible modes of transformations to active carcinogens could be important: formation of carbonyl products directly or via epoxide intermediates and formation of carbocations from the epoxides. If carbonyl products are the ultimate carcinogens, then our results imply that their extent of formation by isomerization from primary products of *P*-450 oxidation rather than their electrophilicities are discriminating factors in determining the relative carcinogenic potency of the parent compounds.

On the other hand, if epoxides themselves can act as ultimate carcinogens without isomerization to carbonyl compounds, then the electrophilicity of their carbocation is a good indicator of relative parent compound activity.

Using the criteria corresponding to all three modes of activation, we predict that 1,2-dichloroethylene which has not yet been studied will be a carcinogen with an activity intermediate between vinylidene chloride and tetrachloroethylene. This prediction remains to be verified.

In screening of other unknown compounds with haloethylene functional groups, the four promising indicators of carcinogenic activity identified here, i.e., isomerization energies with and without epoxide intermediates, stability of epoxides relative to parent compound, and the net charge on the epoxide carbocation should be calculated to further sort out their relative importance and predictive capabilities.

In addition to providing a possible screening procedure for carcinogenic activity relative to vinyl chloride, the results of our studies provide some insight into the mechanism of formation of the ultimate carcinogens. These may be summarized as follows:

(1) The first step in oxidative metabolism of chloroethylenes does not determine the extent of metabolism, consistent with other mechanistic studies [22,23].

(2) Non-enzymatic formation of carbonyl products from the initial products of oxidative metabolism of parent compounds is very probable. Such products can be formed by a radical mechanism at the substrate binding site or from epoxides in a polar environment. Our results are consistent with *both* of these mechanisms.

(3) Formation of an epoxide (Pathway II) could be a critical step in extent of metabolism and formation of the ultimate carcinogen. Experi-

mental data implicating the epoxide as a metabolite mostly using epoxide hydrase as a potential inhibitor of carbonyl products and adduct formation are indirect and incomplete, but this possibility has not been ruled out. Our results offer some corroboration for the involvement of epoxides in activating transformations of the parent chloroethylenes.

CONCLUSION

Properties of six chloroethylenes intermediates and putative ultimate carcinogens which could serve as indicators of their relative metabolic behavior and carcinogenic activity have been calculated using MNDO, a semiempirical, all valence electron, molecular orbital method. Possible pathways of transformation of parent compounds to acylchlorides, chloroaldehydes and epoxides, their putative ultimate carcinogens, were considered, and heats of formation and relative stabilities of intermediates were calculated. Our results indicate that carbonyl compounds could be formed with and without the intermediacy of epoxides, suggesting the possibility of more than one pathway in activation of parent compounds.

The results obtained suggest that if carbonyl products are the active carcinogens, the relative extent of metabolism to these ultimate carcinogens, rather than their electrophilicity is a determinant of the relative carcinogenic activity of the parent compound. Of the various thermodynamic criteria investigated, three involving further transformations of initial products of oxidative metabolism to epoxide and carbonyl products were found to be indicators of both relative metabolic behavior and carcinogenic activity.

The quantities identified in this study as reasonable indicators of the rank order of carcinogenicity of the four known chloroethylenes could be useful as predictors of at least the qualitative carcinogenic activity of unknown compounds with haloethylene functional groups.

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