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Quantum Chemical Studies of the Metabolism of the Inhalation Anesthetics Methoxyflurane, Enflurane, and Isoflurane

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SUMMARY

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Biodegradation of the inhalation anesthetics methoxyflurane, enflurane, and isoflurane is thought to occur by oxidative *O*-dealkylation and dechlorination. In an attempt to understand their known metabolic behavior, we have calculated the conformational and electronic properties of the three compounds and proposed intermediate metabolites using two semiempirical molecular orbital methods, iterative extended Hückel theory and perturbative configuration interaction using localized orbitals. The conformational similarities obtained for the three compounds pointed to electronic properties as the main factors involved, and using these we were able to establish criteria which could account for their known relative extent of metabolism and the formation of a number of identified intermediates.

INTRODUCTION

Until recently, methoxyflurane (Penthrane, $CH_3OCF_2CHCl_2$) has been an extensively used general anesthetic. Concern as to its nephrotoxicity has stimulated a number of studies on its metabolism and it has been shown to be extensively biodegraded (1–4). Studies on rat liver slices *in vitro* have shown it to biodegrade through the processes of enzymatic dechlorination and *O*-dealkylation (ether bond cleavage), requiring both NADPH and oxygen. Recent studies in man, using tracer doses of ^{14}C-labeled methoxyflurane, confirm the earlier animal studies: 7–21% of the methoxyflurane administered underwent ether cleavage and in one subject an additional 40% was dechlorinated. In these studies the identified metabolites of methoxyflurane included dichloracetic acid, methoxydifluoroacetic acid, and free fluoride ion (3). Additional reports confirm the presence of increased amounts of inorganic fluoride and oxalic acid in the sera of patients undergoing methoxyflurane anesthesia and have shown these increases to correlate with renal toxicity (1–6).

Because of the substantial extent of metabolism of methoxyflurane and the apparent toxic effects of its metabolites, two new, structurally related anesthetics, the isomers enflurane (Ethrane, $CHF_2OCF_2 \cdot CHClF$) and isoflurane (Forane, $CHF_2 \cdot OCHClCF_3$), are presently under clinical investigation. A recent study of isoflurane

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(7) under clinical anesthetic conditions demonstrated that it is metabolized to about 20% of the extent of methoxyflurane in both rats and man, based on a determination of ionic fluoride in urine. Organic fluoride was also detected and tentatively identified as trifluoroacetic acid. These results supersede earlier work (8) in which no evidence of isoflurane could be detected in the portal drainage in liver perfusion studies in miniature swine. A comparison of the renal effects of isoflurane and methoxyflurane in rats has also been made (9). Similar recent investigations of the metabolism of enflurane (10, 11) indicate that the extent of metabolism in rats and man is approximately 33% that of methoxyflurane, based on total urinary fluoride determination. Thus, when administered under clinical anesthetic conditions, the relative extent of total metabolism of the three drugs is in the order methoxyflurane > enflurane > isoflurane, and all three metabolize to a significant extent. It should be noted that the metabolism of methoxyflurane is inducible by phenobarbitol while that of enflurane and isoflurane is not (10). Unlike methoxyflurane, oxalic acid was not detected in significantly elevated amounts in the metabolism of enflurane (10), and no fluoride-containing intermediate products of metabolism were identified. However, organic fluoride metabolites were detected in amounts 5 times greater than inorganic fluoride (10). This is strongly suggestive of the presence of such metabolites as fluorochloracetic acid and difluoromethoxydifluoroacetic acid, analogues of the fluoride-containing compounds detected in methoxyflurane metabolism.

The blood/gas and lipid/gas partition coefficients of the three anesthetics are known (12), and methoxyflurane is approximately 10 times more soluble in both blood and lipid than are enflurane and isoflurane. This increased solubility is reflected in the fact that much smaller doses of methoxyflurane are required for clinical anesthesia; i.e., the minimum alveolar concentration (13) for methoxyflurane is less than for enflurane or isoflurane. Since the metabolic studies were conducted using equivalent clinical doses of each anesthetic, (minimum alveolar concentration × hours), this implied an equal molar concentration of the agent at the site of action, i.e., in the lipid phase of the nerve cell. Thus the relative extent of metabolism observed for the three compounds does not reflect differences in lipid solubility which are already accounted for, provided we make the reasonable assumption that the extent of solubility of the agent in the membrane of the nerve cell at the site of action is very similar to that in the membrane which binds the metabolizing enzymes. Further evidence that differences in lipid solubility alone cannot account for differences in extent of metabolism is the fact that enflurane and isoflurane have approximately the same oil/gas partition coefficients (99 vs. 98.5) (12) and yet their metabolic behavior is significantly different.

PROPOSED METABOLIC PATHWAYS AND SUBSTRATES SELECTED FOR STUDY

Two parallel pathways for the biodegradation of methoxyflurane have been derived, as shown in Fig. 1, on the basis of isolation of metabolic intermediates and the known presence of ether-cleaving and dechlorinating enzyme systems (4). Although much less is known about metabolites of enflurane and isoflurane, we propose pathways of biodegradation for these anesthetics in Figs. 2 and 3, which are similar to those established for the structurally similar methoxyflurane.

The first step in pathway I is an enzyme-mitigated ether cleavage, leading for methoxyflurane (Fig. 1) and enflurane (Fig. 2) to an unstable 1-dihaloethyl alcohol which proceeds spontaneously to the formation of the dihaloacetic acid with loss of 2 moles of inorganic fluoride. In methoxyflurane dichloracetic acid has been detected. In enflurane the analogous compound, chlorofluoroacetic acid, is implied by the extensive amount of organic fluoride found (10). In this pathway a second enzymatic step, dehalogenation of the two dihaloacetic acid intermediates, is postulated, leading to the formation of oxalic acid. Since significant amounts of oxalic acid have not as yet been detected in enflurane studies, and elevated amounts of organic fluoride metabolite were present, it is possible that this second step proceeds much less in enflurane than in methoxyflurane. By contrast, the metabolite produced

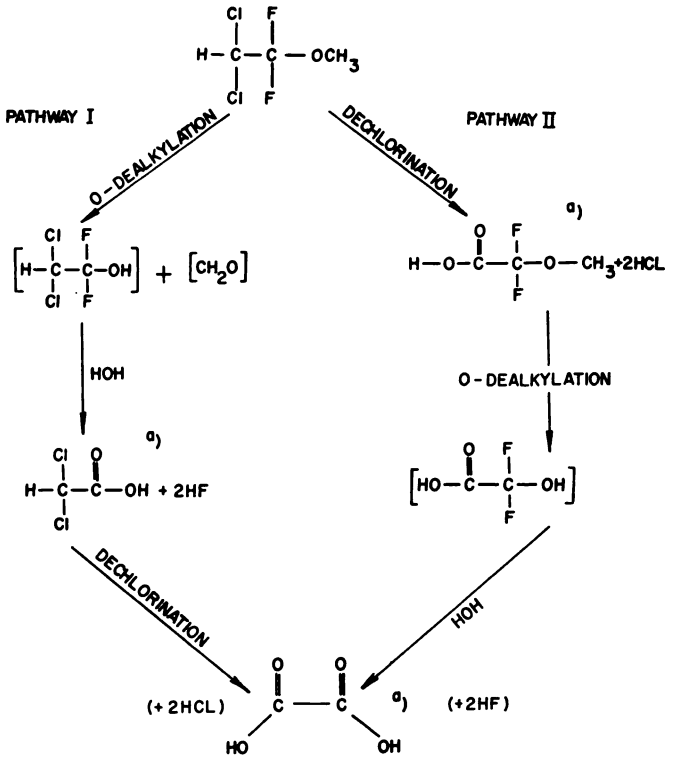


FIG. 1. *Proposed metabolic pathways for the anesthetic methoxyflurane*

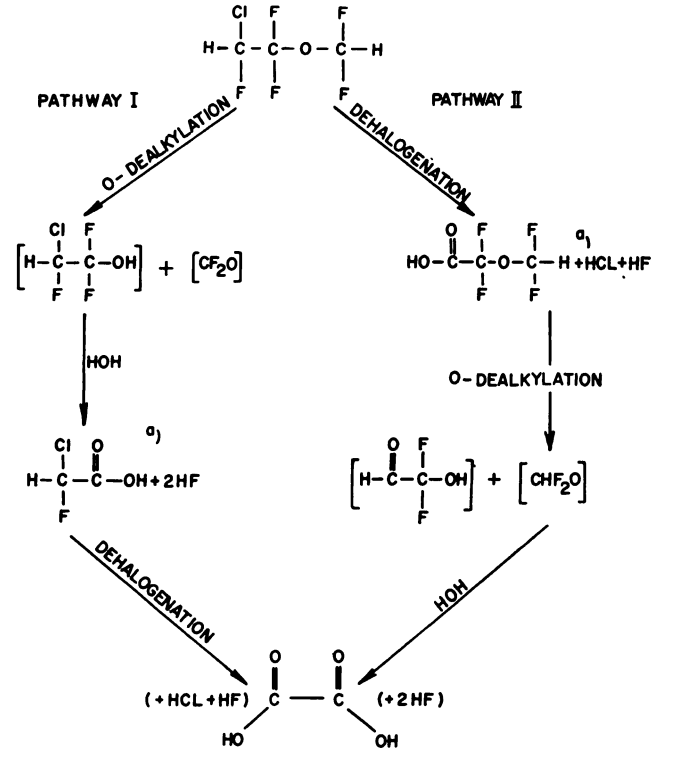


FIG. 2. *Proposed metabolic pathways for the anesthetic enflurane*

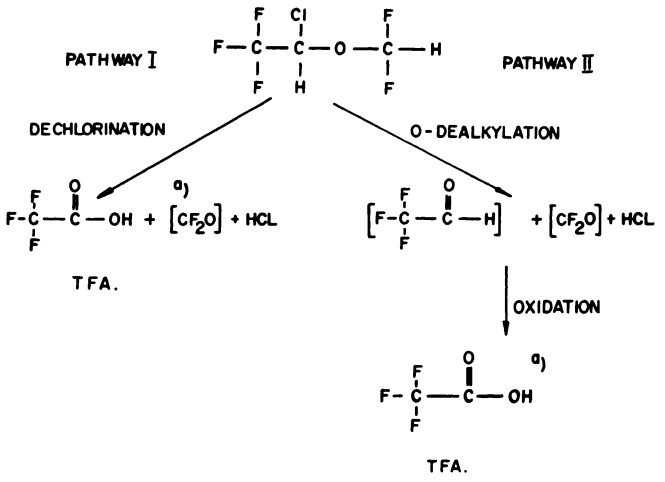


FIG. 3. *Proposed metabolic pathways for the anesthetic isoflurane*
TFA, trifluoroacetic acid.

by ether bond cleavage in isoflurane is trifluoroacetic acid, which is now known to be an end product (7).

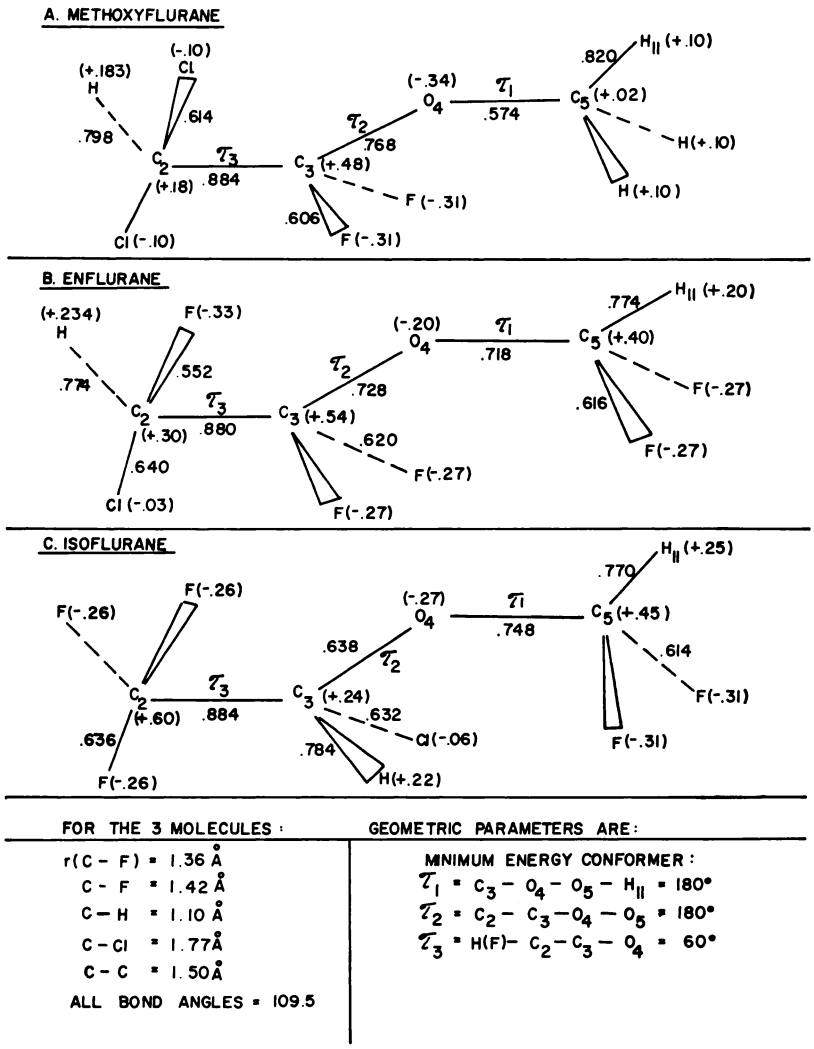
We have made energy conformation studies and calculations of the electronic properties of methoxyflurane, enflurane, and isoflurane to determine molecular properties of the original compounds relevant to ether cleavage behavior. We have also studied the acid intermediates of pathway I, $HCl_2 \cdot CCOOH$ and HClFCCOOH, to try to predict their efficacy as substrates in dechlorination.

In an alternative metabolic pathway proposed for each anesthetic (pathway II, Figs. 1–3), the first step postulated is enzymatic dechlorination. For methoxyflurane and enflurane this step leads to a methoxyhaloacetic acid. In methoxyflurane this intermediate (Fig. 1), methoxydifluoroacetic acid, has been detected. In enflurane the corresponding compound, difluoromethoxydifluoroacetic acid (Fig. 2), contains 4 atoms of fluorine per mole and could be a likely source of the excess amount of organic fluoride found in enflurane studies (10). These intermediates could then serve as substrates for enzymatic ether bond cleavage, leading directly to the formation of oxalic acid. Again, as in pathway I, such subsequent metabolism of an intermediate appears to be much less extensive for enflurane than for methoxyflurane. For isoflurane (Fig. 3) the product of dechlorination would again be trifluoroacetic acid, a known metabolite (7).

To investigate this pathway, in addition to the three original compounds, calculations were also made for the two intermediates which could be substrates of the ether-cleaving enzyme system.

METHOD OF CALCULATION AND BASIC RESULTS

For each compound of interest, the electron distribution, bond density, net atomic charges, and population of atomic orbitals were calculated by a molecular orbital program based on the iterative extended Hückel theory (14, 15). In addition, the energy of each anesthetic molecule as a function of a complete set of nested rotations about all single bonds in all three anesthetic molecules was determined using another approximate molecular orbital method, perturbative configuration interaction using localized orbitals (16). The molecules were found to have the same minimum energy conformer and similar flexibilities. Hence in general there appeared to be no conformational factors which would obviously influence the relative degree of metabolism by a set of rather nonspecific enzymes. Additionally, the electronic properties of the molecules were found to be independent of the rotational conformation. Thus differences in the electronic properties among these compounds, rather than con-



FOR THE 3 MOLECULES :

r(C - F) = 1.36 Å
C - F = 1.42 Å
C - H = 1.10 Å
C - Cl = 1.77 Å
C - C = 1.50 Å
ALL BOND ANGLES = 109.5

GEOMETRIC PARAMETERS ARE:

MINIMUM ENERGY CONFORMER :
$\tau_1 = C_3 - O_4 - O_5 - H_{II} = 180°$
$\tau_2 = C_2 - C_3 - O_4 - O_5 = 180°$
$\tau_3 = H(F) - C_2 - C_3 - O_4 = 60°$

FIG. 4. *Minimum energy conformers, calculated net atomic charges, and bond densities for methoxyflurane, enflurane, and isoflurane*

Numbers with signs in parentheses are the net charges, and numbers between atoms are the fraction of electron density localized between those two atoms, called bond densities.

formational differences, should be primarily responsible for the observed differences in metabolic behavior. The one instance in which conformational consideration might play a role is in the dechlorination of isoflurane, since the chlorine in this isomer is on the α- rather than the β-ethyl carbon atom. Thus, even though the over-all conformation of isoflurane is analogous to enflurane and methoxyflurane, the position of the H—C—Cl group relative to the active site of the metabolizing enzyme would be different in the former substrate.

Figure 4 gives the net atomic charges and bond densities calculated from a Mulliken population analysis (17) of the iterative extended Hückel theory results for each anesthetic molecule. In addition, we list the geometric parameters found for the minimum energy conformation. In methoxyflurane the methyl carbon atom is almost neutral, while in enflurane and isoflurane it

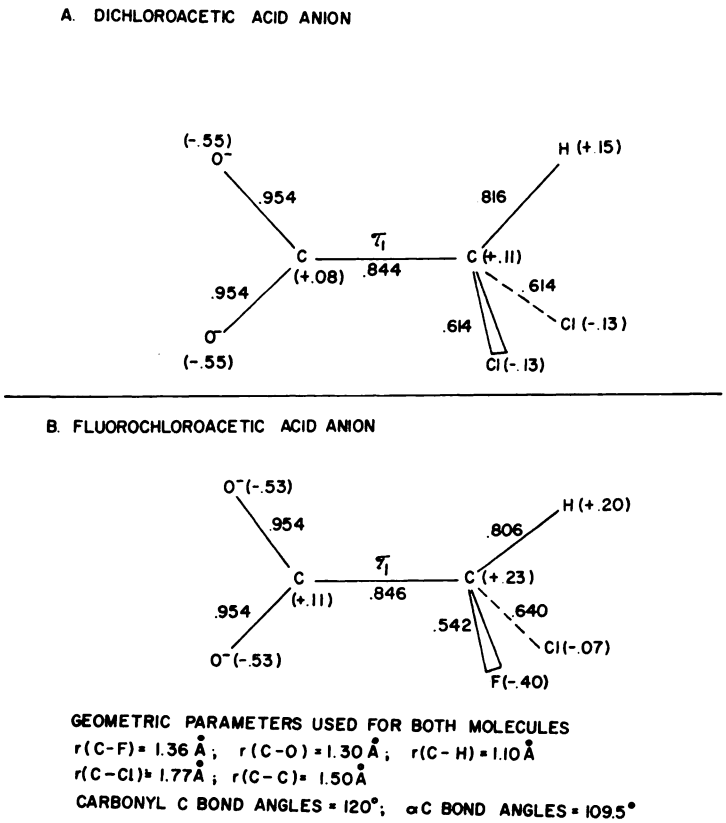


FIG. 5. *Geometric structures, bond densities, and net charges calculated for intermediate metabolites of methoxyflurane (dichloracetic acid) and enflurane (fluorochloracetic acid) by pathway I*
Calculations were made for the acid anions.

has a large positive charge. The positive charge on the β-carbon increases in the order methoxyflurane < enflurane < isoflurane, while the positive charge on the α-carbon is in the order enflurane > methoxyflurane > isoflurane. The hydrogen atoms in methoxyflurane are less positive than those in either enflurane or isoflurane. The bond densities given in Fig. 4 are a measure of the amount of electron density localized between the atoms forming a bond. They can be taken as an approximate measure of the bond strength. We note that in each molecule the most dense bond is the unique C—C bond. The O—CH_3 bond densities increase in the order methoxyflurane < enflurane < isoflurane. The C—Cl bond density in methoxyflurane is less than in either of the other two compounds.

In a similar fashion, Fig. 5 gives the net atomic charges and bond densities for the two haloacetic acid anion intermediates of pathway 1 assumed to be substrates for enzymatic dechlorination. We see from this figure that the net negative charge on the molecule is distributed equally and almost completely on the two oxygen atoms. The remainder of the atoms in the molecule have relatively small net charges, summing almost to a neutral charge. In these acid anions the C—O bonds are equivalent and are the strongest in the molecule, reflecting some double bond character, as would be expected in a carboxyl anion. The α-carbon and hydrogen atoms are more positive in the metabolite derived from enflurane than from methoxyflurane.

Figure 6 gives the net atomic charges and bond densities for the two methoxyhaloacetic acid anion intermediates, the proposed substrates of enzymatic *O*-demethylation in pathway II. Again, as in the ionized acids in

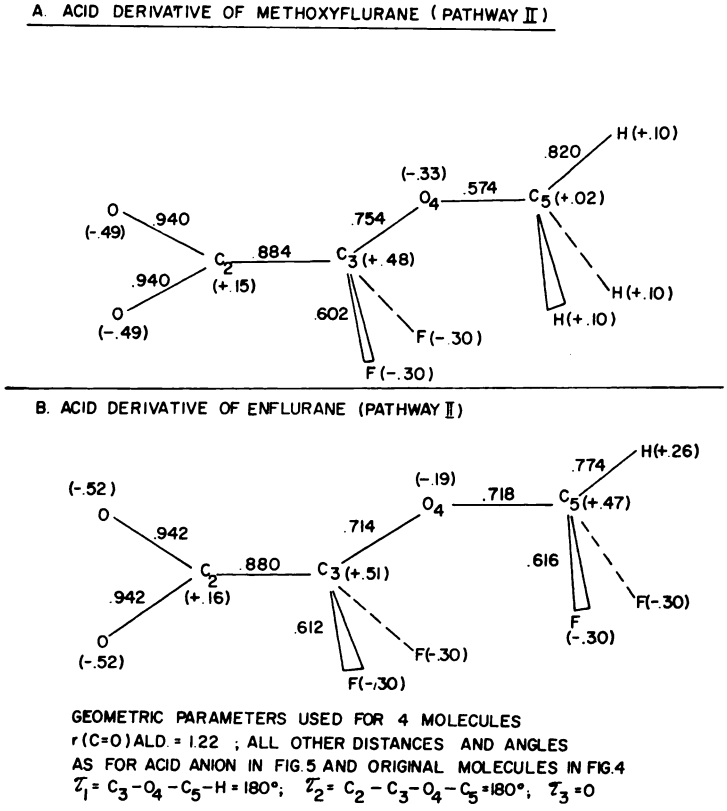


FIG. 6. *Geometric structures, bond densities, and net charges calculated for possible intermediate metabolites of methoxyflurane and enflurane by pathway II*

Fig. 5, the carboxyl oxygens share the negative charge equally; the two C—O carboxyl bonds are equivalent and are the strongest in the molecule. The O—CH_3 bond is weaker, and the methyl carbon and hydrogen atoms are less positive, in the metabolite derived from methoxyflurane than in that from enflurane.

CORRELATION OF RESULTS WITH METABOLIC BEHAVIOR

Metabolic Mechanisms

The two enzymatic processes which appear to be involved in the biodegradation of the three volatile anesthetics under study here are *O*-dealkylation and dehalogenation. There is general agreement among active investigators in this field that *O*-dealkylation proceeds by hydroxylation of the carbon atoms *alpha* to the oxygen atom. It is further thought that this hydroxylation occurs by the insertion of an active oxygen atom, bound as a ligand to cytochrome P-450, into a C—H bond. Some evidence for this mechanism of *O*-dealkylation is the observation that O_2 and NADPH are universally required as cofactors, and studies using $^{18}O_2$ showed incorporation of ^{18}O into the leaving methyl group (18); *O*-dealkylation of 4-nitroanisole showed an isotope effect (K_H/K_D) = 2, indicating the involvement of the C—H or C—D bond in the enzymatic process (19). Studies with model systems have also indicated the above mechanism (20). A great deal of speculation has revolved around the question of the nature of the "active oxygen," with no definitive resolution of this key question at the present time. The current hypothesis, however, is that the oxygen, while still in the molecular state and bound to the iron of the activating enzyme, has an oxene or oxenoid character, rendering it electron-deficient and isoelectric with car-

benes. Insertion of this oxygen atom would then represent an electrophilic attack of the oxygen on the carbon or the hydrogen atom of the C—H bond, or both.

Unlike *O*-dealkylation, dehalogenation may require a number of possible enzymatic processes. Three of these involve some sort of nucleophilic attack on the carbon atom to which the leaving halogen is attached. One such process involves a soluble enzyme which catalyzes the transfer of the sulfhydryl group of a reduced glutathione to the alkyl moiety of alkyl halide substrates (21, 22). Another observed process is reductive dechlorination, which appears to be involved in the metabolism *in vitro* of dichlorodiphenyltrichloroethane in pigeon liver, as well as that of CCl_4 and possibly halothane and other chlorinated hydrocarbons. Evidence points to a mechanism involving reduced cytochrome P-450 or cytochrome b_5 and the binding of the alkyl halide to the ligand position (23–28). Finally, enzymes have been found which catalyze the hydrolysis of a carbon-halogen bond. Three such halidohydrolases have been found thus far, all in a pseudomonad (29). All three of these processes could involve a nucleophilic attack on the relatively electron-deficient carbon atom to which the leaving halogen is attached. The glutathione reaction involves the transfer of a sulfhydryl group to that carbon atom, while reductive dechlorination involves the transfer of 2 electrons to it, and during hydrolysis an OH^- group attaches to the carbon atom. In a previous theoretical study of the nine chlorinated ethanes, we did indeed find a correlation between the extent of dechlorination and the degree of electron deficiency of the carbon atom (30).

There is also evidence for a mechanism of enzymatic dechlorination in mammals requiring molecular oxygen and NADPH and implicating membrane-bound cytochome P-450 (31). It is thus possible that such dechlorination proceeds by the insertion of an active, electron-deficient "oxene" oxygen into the C—H bond of the carbon to which the chlorine is attached, followed by the spontaneous conversion of an HO—C(—Cl)< group to >C=O. Depending on whether there is another halogen attached to the same carbon, an aldehyde (ketone) or carboxylic acid would be formed. This mechanism is consistent with but not necessarily proven by the detection of the intermediate metabolite, $HOOC—CF_2—O—CH_3$, in the metabolism of methoxyflurane.

Much of the above speculations concerning the pathway and mechanism of degradation of the halogenated ethers remains to be verified. Nevertheless, the results of our stereoelectronic description of the compounds which are substrates for both dechlorinating and *O*-dealkylating enzymes, together with what is known of the metabolic behavior of the three anesthetics, should permit us, on the one hand, to test the plausibility of the proposed mechanisms and, on the other hand, to test the efficacy of our methods to predict the relative metabolic behavior of these related compounds.

Ether Bond Cleavage

Molecular factors influencing ether bond cleavage. Assuming that ether bond cleavage proceeds by the insertion of an electron-deficient active oxygen into the $C_{methyl}—H$ bond and subsequent cleavage of the hemiketal bond, we postulate that the substrate properties most crucial in determining the relative extent of this reaction are (a) the relative nucleophilicity of the carbon atom, (b) the relative nucleophilicity of the hydrogen atom, and (c) the relative strength of the C—H bond. As a measure of the relative bond strength we use the calculated values of bond densities. As a measure of the relative nucleophilicity of the carbon atom we propose two main criteria: the net charge on the carbon atom, and the electron density in the carbon atomic orbital binding to the hydrogen atom, and possibly the energy of these electrons which are involved in donation to the active oxygen. For the hydrogen atom the net charge and number of electrons in its bond to the carbon atom are the parallel properties to consider.

Ether cleavage, pathway I. Table 1A contains the calculated molecular properties

TABLE 1

Correlation of electronic properties with extent of metabolism by pathway I

A. O-Demethylation of anesthetics

Compound	$q_{C\text{-methyl}}$[a]	$q_{H\text{-methyl}}$[a]	ρ_{C-H}[b]	ρ_H[c]	$\rho_C(H)$
Methoxyflurane	+0.02	+0.10	0.820	0.90	0.804[d] (+0.26)[e]
Enflurane	+0.40	+0.20	0.774	0.80	0.748 (+0.25)
Isoflurane	+0.45	+0.25	0.770	0.75	0.744 (+0.24)

B. Dechlorination of intermediate $[XClHC_\alpha—COO^-]$

Compound	$q_{C\alpha}$[a]	q_H[a]	ρ_H[c]	$\rho_H(H)$	ρ_{C-H}[b]	$\rho_C(Cl)$	ρ_{C-Cl}[b]
X = Cl (methoxyflurane)	+0.11	+0.15	0.85	0.706[d] (+0.27)[e]	0.826	0.62[f] (+0.27)[g]	0.614
X = F (enflurane)	+0.23	+0.20	0.90	0.651 (+0.26)	0.806	0.65 (+0.26)	0.640

[a] q = net charge in fraction of electron unit.

[b] ρ_{A-B} = electron density in bond between atoms A and B.

[c] ρ_H = electron density in H atomic orbital binding to C.

[d] $\rho_C(H)$ = p electron density in C atomic orbital binding to H.

[e] Numbers in parentheses = s electron density in C atomic orbital binding to H.

[f] $\rho_C(Cl)$ = p electron density in C atomic orbital binding to Cl.

[g] Numbers in parentheses = s electron density in C atomic orbital binding to Cl.

just discussed as good criteria for determining the relative extent of enzymatic ether bond cleavage (O—CH_3) in the three anesthetics. There is a striking increase in the net positive charge both on the methyl carbon atom and, to a lesser extent, on the hydrogen atom, in going from methoxyflurane to enflurane. This trend continues to some extent in isoflurane. Whatever the detailed mechanism of the insertion of the electrophilic oxygen atom, it would certainly be less likely to occur as the carbon and hydrogen atoms themselves become more positive. Thus we have obtained a rather definitive correlation which would predict that *O*-dealkylation occurs to a much greater extent in methoxyflurane than in either enflurane or isoflurane.

From Table 1A we also note that as the carbon and hydrogen atoms become more positive, the number of electrons in their bond (ρ_{C-H}) also decreases. This decrease could be taken as a measure of the weakening of the C—H bond. Thus, if C—H bond cleavage were the initiating step in the insertion, the correlation obtained with extent of metabolism would be inverted. Our results clearly implicate interaction with the active oxygen as the important first step which initiates the C—H bond cleavage, and not the other way around. If it occurs, variation in the net charge on the atoms themselves which are involved in electron donation would appear to be a more determining characteristic for extent of insertion than the C—H bond density.

In the last column of Table 1A is listed the fraction of p electron density in the carbon orbital binding to the hydrogen in the three compounds (ρ_C). By this criterion, again, the order of ability to donate electrons to the oxygen would be methoxyflurane > enflurane > isoflurane, predicting the same relative extent of *O*-dealkylation as the net charge variation. We have particularly singled out the p electron density as the likely candidate for incipient charge transfer, since these are the most energetic electrons involved in the C—H bond and would require the least energy to transfer. The figures in parentheses in the same column of Table 1A are the amounts of the more stable 2s electrons involved in bonding.

If the hydrogen atom is also involved in electron donation to the oxygen, then the greater electron density in its atomic orbital, the better donor it would be. We see from both the second ($q_{H\text{-methyl}}$) and fourth (ρ_H)

TABLE 2

Correlation of electronic properties with extent of metabolism by pathway II

A. Dechlorination of anesthetics

Compound	q_C[a]	q_H[b]	ρ_H[c]	ρ_C(H)	ρ_{C-H}[f]	ρ_C(Cl)	ρ_{C-Cl}[f]
Methoxyflurane	+0.18 (β)	+0.18	0.82	0.705[d] (+0.26)[e]	0.798	0.65[g] (+0.26)[h]	0.614
Enflurane	+0.30 (β)	+0.23	0.77	0.648 (+0.26)	0.774	0.66 (+0.26)	0.640
Isoflurane	+0.24 (α)	+0.22	0.78	0.702 (+0.25)	0.784	0.71 (+0.25)	0.632

B. O-Demethylation of intermediate [$XCH—O—CF_2—COO^-$]

Compound	$q_{C\text{-methyl}}$	$q_{H\text{-methyl}}$	ρ_H[c]	ρ_C[d](H)
X = H (methoxyflurane)	+0.02	+0.10	0.90	0.86 (+0.26)[e]
X = F (enflurane)	+0.47	+0.26	0.74	0.80 (+0.26)

[a] q_C = net charge on C atom (α or β) with Cl substituent.
[b] q_H = net charge on H atom on C atom (α or β) with Cl substituent.
[c] ρ_H = electron density in H atomic orbital binding to C.
[d] ρ_C(H) = p electron density in C atomic orbital binding to H.
[e] s electron density in C atomic orbital binding to H.
[f] ρ_{A-B} = electron density in bond between atoms A and B.
[g] ρ_C(Cl) = p electron density in C atomic orbital binding to Cl.
[h] s electron density in C atomic orbital binding to Cl.

columns that by this criterion as well, the extent of *O*-dealkylation would be methoxyflurane > enflurane > isoflurane.

Cleavage of intermediates in pathway II. As can be seen in Fig. 3, the dechlorination of isoflurane leads directly to trifluoroacetaldehyde and by oxidation to trifluoroacetic acid, as in pathway I. For methoxyflurane and enflurane, the product of dechlorination, a methoxyhalo acid, could be a substrate for *O*-dealkylation.

We have already discussed the molecular properties used to monitor the ease of ether cleavage. Table 2B gives the values calculated for these factors relevant to O—CH_3 cleavage for the two indermediates. In these molecules exactly the same criteria were used for each O—CH_3 cleavage as for parent molecules themselves. The net positive charge on the carbon and hydrogen atoms is greater; the number of carbon $2p$ electrons in the carbon orbital binding to hydrogen is less, as is the electron density in the hydrogen orbital binding to the carbon.

All these factors lead to the conclusion that the intermediate of methoxyflurane metabolism would be the more reactive substrate in ether cleavage rather than the intermediate from enflurane metabolism.

Dechlorination

Molecular factors affecting dechlorination. If dechlorination proceeds by the insertion of an electron-deficient "active" oxygen atom into a C—H bond of the carbon to which the leaving chlorine is attached, a similar criterion for the extent of dechlorination would prevail, as in the case of O—CH_3 cleavage. Thus criteria for nucleophilicity of the carbon and hydrogen atoms would be relevant. The more positive each atom and the less electron density in the bonding atomic orbitals connecting the two, the less easy will be insertion of the electron-deficient oxygen. Since the C—Cl bond does cleave, if it is at all concerted with oxygen insertion, the strength of this bond would also be a factor. Dechlorination can also proceed through at least three other mechanisms, all of which involve nucleophilic attack on the carbon atom to which the chlorine is attached. Thus it is also possible that properties of this same carbon which pertain to the extent of its electron deficiency would be important. In our previous study the most relevant measure of the electrophilicity of the carbon atom in any reaction in which the Cl^- would be displaced appeared to be the degree of elec-

tron deficiency in the carbon atomic orbital bonding to the leaving Cl^-. In our current study we have examined both sets of criteria—those relevant to active oxygen insertion and those relevant to Cl^- displacement—in our attempt to predict the relative extent of dechlorination of the three anesthetics and their metabolites and also gain insight into the mechanism of dechlorination.

Dechlorination of intermediates of pathway I. As shown in Figs. 1–3, the stable metabolite which is the result of ether cleavage for all three anesthetic molecules is a haloacetic acid. The trifluoroacetic acid formed by isoflurane at this stage would be an end product. For the other anesthetics, the haloacetic acids contain chlorine and could serve as substrates for a dechlorinating enzyme system. Such a dechlorination would be the second key reaction in determining the total extent of biodegradation possible by pathway I. Table 1B presents the properties relevant to the extent of dechlorination of dichloracetic acid and chlorofluoroacetic acid (intermediates of methoxyflurane and enflurane, respectively).

All the criteria related to insertion—namely, net positive charge on the carbon and hydrogen atoms, and electron density in the carbon orbitals binding to the hydrogen and in the hydrogen orbitals binding to the carbon—predict that the dichloracetic acid from methoxyflurane dechlorinates more readily, leading to greater production of oxalic acid from methoxyflurane than from enflurane (Table 1B). This is in keeping with current clinical observations (4, 10). The variation in C—Cl bond density, $\rho_{C\text{-}Cl}$, could also favor dechlorination of dichloracetic acid if C—Cl bond cleavage were at all concerted with oxygen insertion. The C—H bond density criterion again appears to be superseded by the decreased nucleophilicity of the carbon and hydrogen atoms in going from the dichloro- to the chlorofluoroacetic acid. On the other hand, if one considers Cl^- displacement, the degree of electron deficiency in the carbon atomic orbital binding to the leaving chlorine decreases only slightly from the dichloro- to the chlorofluoroacetic acid. Thus the diminished metabolism of the latter would be negligible by this mechanism compared to an insertion mechanism. The fact that very little oxalic acid is observed in enflurane metabolism, together with the criteria we have used to test each possibility, favor an oxygen insertion mechanism for dechlorination.

Initial dechlorination: pathway II. As shown in Figs. 1–3, dechlorination of the anesthetic molecules is postulated as the first step in biodegradation along pathway II. Table 2A contains all the calculated molecular factors just described, which could play a role in determining the extent of dechlorination of these three molecules. We see from the first four columns of this table that if dechlorination proceeds by an active electron-deficient oxygen insertion into the C—H bond of the carbon to which the chlorine is attached, the increasing positive charge on both carbon and hydrogen and the decrease in electron density in their bonding atomic orbitals would favor the dechlorination of methoxyflurane over enflurane or isoflurane. These electronic criteria alone would predict that isoflurane should dechlorinate to a somewhat greater extent than enflurane. However, unlike both methoxyflurane and enflurane, the chlorine atom in isoflurane is on the α- rather than the β-ethyl carbon atom. Analogous minimum energy conformers were found for all three compounds. Thus the H—Cα—Cl group in isoflurane must be in a sterically different position relative to the active oxygen in the dechlorinating enzyme than is the H—Cβ—Cl group of the other two molecules. The different relative arrangement of the transferring oxygen and the group to which it is transferred could easily play a role in determining the efficacy of the transfer and might diminish it. Again the variation in density of the C—H bond in all three compounds appears either not to play a role or to be superseded by the variation in nucleophilicity of the carbon and hydrogen atoms. To investigate dechlorination by direct displacement of the Cl^-, Table 2A also lists the number of p electrons in the carbon atomic orbital binding to the leaving chlorine. By this criterion, the extent of dechlorination of methoxyflurane and enflurane would be about equal to each other and greater than that of isoflurane. The variation in C—Cl

bond strength favors methoxyflurane dechlorination, if the C—Cl cleavage occurs with any degree of concertedness in either mechanism.

Degree of Competitiveness of Pathways I and II for Each Molecule

In addition to predicting the relative extent of metabolism of the three anesthetic molecules to be expected by each proposed pathway, we can use the criteria we have established for *O*-dealkylation and dechlorination to predict the extent to which each molecule would initially follow pathway I vs. pathway II in its biodegradation. Comparing the calculated properties listed in Table 1A and B, we obtain the interesting result that pathway I would be greatly favored over pathway II in methoxyflurane, with the reverse situation holding for the other two compounds.

CONCLUSIONS

From our molecular orbital analysis of the electronic features of methoxyflurane, enflurane, and isoflurane, we have deduced that the order of susceptibility of these drugs to initial ether bond cleavage or dechlorination is methoxyflurane > enflurane > isoflurane. Moreover, we have deduced that pathway I is favored in methoxyflurane metabolism while pathway II would be favored in enflurane and isoflurane metabolism. We have also deduced that the intermediate metabolites formed by either pathway I or II of methoxyflurane would be better substrates for continuing enzymatic metabolism than their counterparts in enflurane. These deductions are in keeping with and help to elucidate the known behavior of these anesthetics up to the present. For example, our results are consistent with the reduced amount (20%) of inorganic fluoride detected from the metabolism of isoflurane compared to methoxyflurane. Also, if we consider the quantitative limit of our qualitative predictions for enflurane metabolism, i.e., that 100% of the metabolism of enflurane proceeds by pathway II and the intermediate formed is almost completely inactive as a substrate for *O*-dealkylation, we would predict a ratio of inorganic fluoride to organic fluoride of 1:4, with no oxalic acid formed in enflurane metabolism. This result is in striking accord with findings in the most recent study of enflurane metabolism (10) and could be further corroborated by the identification and measurement of the amounts of organic, fluoride-containing metabolites. We also predict greater accumulation of difluoromethoxydifluoroacetic acid from enflurane than of methoxydifluoroacetic acid from methoxyflurane, a result that could also be verified by further studies. More inorganic fluoride is obtained from methoxyflurane than from enflurane metabolism, despite the fact that 4 moles of inorganic fluoride would be released from each pathway of enflurane compared to 2 for methoxyflurane metabolism. In view of the number of moles of inorganic fluoride released in each step of each proposed pathway (Figs. 1 and 2), these results are in keeping with our prediction that pathway I is favored in methoxyflurane metabolism and proceeds to a much greater extent than in enflurane, while in enflurane metabolism pathway II is favored and the initial step would proceed to a greater extent than the second step.

The criteria we have established for ease of *O*-dealkylation are based on the assumption of an active oxygen insertion mechanism. Correlation with observed behavior is best if we also assume that dechlorination proceeds by a similar mechanism. The main aspect of our correlation is that insertion of active oxygen into C—H bonds is more likely to occur, the more electron-rich each atom is. Insofar as the assumption of these mechanisms, coupled with our analysis, leads to agreement with observation and correct predictions, we have increased the plausibility of these postulated but not proven mechanisms.

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