Factors Affecting the Formation of Chlorotrifluoroethane and Chlorodifluoroethylene from Halothane

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Since CF₂CH₂Cl and CF₂CHCl are probably the products of reactive intermediates formed during the reductive metabolism of halothane (CF₃CH(CBr)Cl), factors affecting their in vitro and in vivo formation were investigated. In vitro studies with rat hepatic microsomes showed that CF₂CH₂Cl and CF₂CHCl are produced by cytochrome P-450 mediated reductive pathways which were inhibited by the presence of CO. Under conditions of exposure known to promote halothane hepatotoxicity in phenobarbital treated rats (1 per cent halothane, 14 per cent oxygen), the hepatic and blood concentrations of the volatile metabolites were enhanced. Central venous levels of the volatile metabolites were much higher than the concentration in peripheral vessels. The CF₂CH₂Cl/CF₂CHCl ratio in blood was approximately three, whereas the ratio in vitro was almost unity. Liver levels of the two volatile metabolites greatly exceeded the blood levels, but interestingly they were present in equivalent concentrations. The differences in the ratio of CF₂CH₂Cl to CF₂CHCl may be explained by the fact that CF₂CHCl is further degraded under oxidative conditions, whereas CF₂CH₂Cl appears relatively stable. Measurement of these metabolic products in patients undergoing halothane anesthesia may permit rapid detection of an unusually high level of halothane biotransformation along its hepatic pathway. (Key words: Anesthetics, volatile: halothane. Biotransformation, (drug): enzyme induction; fluorometabolites; microsomes. Metabolism: enzyme induction; microsomes. Toxicity: hepatic; metabolites.)

Halothane (CF₃CH(CBr)Cl) is known to undergo oxidative metabolism to the stable end product, trifluoroacetic acid.¹,² There is also evidence that halothane can undergo reductive biotransformation which may be related to its suspected hepatotoxicity.³ Until recently, the only evidence for this reductive pathway was indirect. The evidence included enhanced defluorination and covalent binding of ¹³C-halothane under anaerobic conditions in vitro⁴,⁵ and under hypoxic conditions in vivo.⁶,⁷ However, the discovery of the volatile metabolites of halothane, chlorodifluoroethylene (CF₂CHCl) and chlorotrifluoroethane (CF₃CH₂Cl), in the expired air of rabbits, rats, and humans provided direct evidence that halothane underwent reductive dehalogenation.⁸–¹⁰ Thus, conditions that are known to promote defluorination and covalent binding of halothane reactive intermediates, as well as promote halothane hepatotoxicity, should also enhance the formation of these volatile metabolites.

Using our recently reported head-space gas chromatographic technique,¹¹ a preliminary communication was presented describing the detection of the volatile metabolites in the blood and liver of rats, especially those exposed to halothane in an hypoxic environment.¹² Gourlay et al.¹³ have also reported an increase in the level of the volatile metabolites in the expired air of rats exposed to halothane at reduced FIO₂. Their reported time course for the expiration of these metabolites is similar to our previously reported metabolite profiles in blood.¹¹ Because the nature of these metabolites suggests that they are the end products of proposed reactive intermediates formed during the reductive metabolism of halothane, it is important to know what factors affect their formation and subsequent disposition.

The studies reported here were designed to determine if the factors known to promote halothane hepatotoxicity¹⁴ (microsomal enzyme induction and hypoxia) also promote the formation of CF₂CHCl and CF₂CH₂Cl. In addition, in vitro studies with hepatic microsomes were performed to determine the role of cytochrome P-450 in the formation of these metabolites.

Materials and Methods

Halothane (Ayerst Laboratories, Inc., New York, New York), 2-chloro-1,1-difluoroethylene (CF₂CHCl) and 2-chloro-1,1,1-trifluoroethane (CF₃CH₂Cl) (PCR Research Chemicals, Inc., Gainesville, Florida) were determined by gas chromatography to be 99+ per cent pure. All biochemicals were obtained from Sigma Chemical Co., St. Louis, Missouri. Other chemicals were of reagent grade quality and were used without further purification.

Male Sprague-Dawley rats (150–175 g) were obtained from Hilltop Lab Animals, Inc., Chatworth, California and maintained on a diet of Purina®

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Laboratory Chow and water ad libitum. To induce hepatic biotransformation enzymes, rats were treated intraperitoneally with Aroclor® 1254** (500 mg/kg in sesame oil) four days before use or maintained on 0.1 per cent phenobarbital in drinking water for 10 days prior to anesthesia with halothane or isolation of hepatic microsomes. Control rats received corresponding volumes of sesame oil or tap water.

Control and phenobarbital treated rats were placed into a 180-l Plexiglas® chamber and exposed to 1 per cent halothane for 2 hours. Oxygen concentrations were maintained at 99, 21, or 14 per cent and delivered at a total flow of 6 l/min with nitrogen as the diluent gas. Oxygen levels in the chamber were continuously monitored by a Model 600 oxygen membrane analyzer (Ohio Medical Products, Madison, Wisconsin).

At the end of the halothane exposure, the animals were killed, blood from the inferior vena cava was collected, and the livers were removed. Tail blood from other animals was taken at various times after anesthesia. The blood samples were collected with a heparinized syringe and transferred in 200-μl aliquots into tared 1-ml screw cap microreaction vials with Teflon®-lined rubber septums.** The remaining blood was stored in plastic tubes for fluoride analysis. Livers were weighed and 2.0-g portions were placed into Dounce homogenizers containing 6.0 ml of ice cold 50 mM Tris-KCl buffer (pH 7.4). After homogenization, 200-μl aliquots were transferred to tared 1-ml septum vials. All vials were reweighed for accurate determination of blood and liver aliquots. The recovery and precision of the technique have previously been reported.**

For in vitro studies, rats were killed by cervical dislocation and hepatic microsomes prepared as previously described. Levels of cytochrome P-450 were determined by the method described by Omura and Sato. The resuspended microsomes were adjusted to the desired protein concentration and aliquots were added to 5-ml screw cap microreaction vials. Each incubation (2.5 ml total volume) contained microsomal protein (5 mg/ml) in 50 mM Tris and 154 mM KCl, pH 7.4. Controls were run concurrently using heat denatured microsomes or incubations without the NADPH generating system.

The samples were flushed with O₂, N₂ or pure CO for 10 s and allowed to equilibrate for 10 min. The N₂ samples were purged with N₂ three more times. The samples were then equilibrated by heating at 37°C for 10 min. The reaction was initiated by in-

jacting 2.0 μl (18.8 μmol) of halothane and 100 μl of an NADPH generating system (0.2 mM NADP; 4 mM glucose-6-phosphate; 1 unit of glucose-6-phosphate dehydrogenase; 20 mM MgCl₂) through the septum. The incubations were terminated at 30 min by injecting 20 μl of 6 N HCl into the reaction mixture and analyzed for CF₂CHCl, CF₂CH₂Cl, and F⁻. When indicated the microsomal enzyme inhibitor, SKF-525A, was added to the incubations at a final concentration of 1 mM.

In certain experiments, 50 μmol of CF₂CHCl or CF₂CH₂Cl in dimethylformamide was used as substrate for the microsomal incubations under either an oxygen or nitrogen atmosphere. Reactions were terminated at 30 min by injecting 20 μl of 6 N HCl into the incubation mixture and analyzed for the formation of F⁻ from CF₂CHCl and CF₂CH₂Cl, F⁻, and trifluoroacetic acid from CF₂CH₂Cl.

CF₂CHCl and CF₂CH₂Cl were assayed for by a head-space gas chromatographic method described by Maiorino et al. An aliquot of the head-space of the sample vials, previously equilibrated at 37°C, was injected onto a 6-ft Porapak Q® column operated at 150°C in a Varian 3700 gas chromatograph equipped with FID. The integrated areas of the separated peaks were quantified by means of a standard calibration curve. The standards containing known amounts of CF₂CHCl and CF₂CH₂Cl were prepared as previously reported. Trifluoroacetic acid was measured by utilizing a modification of the head-space procedure of Breimer et al. For trichloroacetic acid. Blood and microsomal samples were diluted 1:1 with total ionic strength activity buffer (Orion Research Incorp., Cambridge, Massachusetts) and the free inorganic fluoride concentration was measured with an Orion® fluoride ion selective electrode.

To determine whether CF₂CHCl and CF₂CH₂Cl are hepatotoxic, phenobarbital-pretreated rats were exposed to these gases under static conditions at 14 per cent O₂ at concentrations similar to those reported by Raventos and Lemon and Torkelson et al. Delivery of CF₂CHCl (26,500 ppm) and CF₂CH₂Cl (3,500 ppm) was performed by bleeding the compressed cylinder at a fixed flow rate into the static chamber. The chamber levels of CF₂CHCl or CF₂CH₂Cl were monitored by gas chromatography using a thermal conductivity detector and a Porapak Q® column operated at 150°C. Tail blood was obtained immediately after exposure for analysis of serum fluoride, CF₂CHCl, and CF₂CH₂Cl. The rats were sacrificed 24 hours after exposure. Serum fluoride was again determined and sections of the livers were preserved in buffered formalin and prepared for histological examination and scored as previously.

** Generously supplied by Monsanto Chemical Co., St. Louis, Missouri.
described. In addition, blood samples were obtained for determination of serum glutamic-pyruvic transaminase activity.

The level of statistical significance was determined by Student’s t test with \( P < 0.05 \) considered significant.

**Results**

In the *in vitro* microsomal studies, the production of CF₂CHCl, CF₂CH₂Cl and F⁻ from halothane were influenced by the atmosphere under which the incubations were performed, and the levels of microsomal cytochrome P-450 (fig. 1). When the incubations were performed under an atmosphere of \( N₂ \), volatile metabolite production and F⁻ release increased as the level of cytochrome P-450 increased. It should be stressed that these cytochrome P-450 levels were measured from the livers of control (0.52 nmol/mg), phenobarbital treated (1.47 nmol/mg) and Aroclor® 1254 (2.65 nmol P-450/mg) treated rats. Incubations performed in an oxygen atmosphere produced only trace amounts of CF₂CH₂Cl, CF₂CHCl, or F⁻ regardless of the cytochrome P-450 content of the microsomes (fig. 1 and table 1).

There was a close stoichiometric relationship between inorganic fluoride release and the production of CF₂CHCl and CF₂CH₂Cl when the incubations were performed under \( N₂ \) (table 1). When CF₂CH₂Cl was incubated with microsomes under an oxygen atmosphere, neither CF₂CHCl (less than 0.1 nmol·mg⁻¹·30 min⁻¹) nor fluoride (less than 0.5 nmol·mg⁻¹·30 min⁻¹) were formed. In addition, incubations of CF₂CH₂Cl under an oxygen atmosphere did not produce measurable trifluoroacetic acid (less than 1 nmol·mg⁻¹·30 min⁻¹) while halothane produced greater than 20 nmol·mg⁻¹·30 min⁻¹ trifluoroacetic acid. However, incubations with CF₂CHCl under an oxygen atmosphere produced prodigious quantities of F⁻ (25.3 nmol·mg⁻¹·30 min⁻¹). Smaller quantities of F⁻ were released from CF₂CHCl when incubations were performed under a nitrogen atmosphere. Thus, CF₂CHCl is not a stable end product under an oxygen atmosphere.

The production of CF₂CHCl, CF₂CH₂Cl and F⁻ from halothane by the microsomal incubations required anaerobic conditions, NADPH, and was in-

**Table 1. Factors Affecting the *In Vitro* Metabolism of Halothane to Chlorodifluoroethylene (CF₂CHCl), Chlorotrifluoroethane (CF₂CH₂Cl), and Inorganic Fluoride (F⁻)†**

<table>
<thead>
<tr>
<th>Condition‡</th>
<th>CF₂CHCl</th>
<th>CF₂CH₂Cl</th>
<th>F⁻</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N₂ )</td>
<td>4.28 ± .34</td>
<td>5.24 ± .70</td>
<td>4.59 ± .37</td>
<td>11</td>
</tr>
<tr>
<td>( O₂ )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.12 ± .01</td>
<td>4</td>
</tr>
<tr>
<td>CO</td>
<td>0.03 ± .02</td>
<td>0.10 ± .05</td>
<td>0.21 ± .02</td>
<td>4</td>
</tr>
<tr>
<td>-NADPH$</td>
<td>0.02 ± .01</td>
<td>0.02 ± .01</td>
<td>0.01 ± .01</td>
<td>5</td>
</tr>
<tr>
<td>+SKF-525A$</td>
<td>4.30 ± .39</td>
<td>4.96 ± 1.17</td>
<td>4.36 ± .92</td>
<td>6</td>
</tr>
<tr>
<td>Denatured</td>
<td>0.01 ± .00</td>
<td>0.02 ± .00</td>
<td>0.22 ± .04</td>
<td>4</td>
</tr>
<tr>
<td>microsomes$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are expressed in units of nmol·mg protein⁻¹·30 min⁻¹.
† Number of separate experiments.
‡ For specific conditions and concentrations see Methods. Concentration of cytochrome P-450 was 1.47 nmol/mg protein in hepatic microsomes from phenobarbital-treated rats. Data represent mean ± SE.
§ Incubations performed under a \( N₂ \) atmosphere.

**Fig. 1. Relationship of cytochrome P-450 levels to the microsomal formation of chlorodifluoroethylene (CF₂CHCl) and chlorotrifluoroethane (CF₂CH₂Cl) from halothane. All values are expressed as the mean ± SE for incubations performed with hepatic microsomes obtained from control (0.52 nmol P-450/mg), phenobarbital (1.47 nmol P-450/mg), or Aroclor® 1254 (2.65 nmol P-450/mg) treated rats. Microsomes were obtained from six rats per treatment group and then pooled for the incubations. Incubations were performed in atmospheres of \( O₂ \) (——) or \( N₂ \) (———) and levels of CF₂CH₂Cl (□), CF₂CHCl (○) and F⁻ (●) were determined after 30 min. Number of experiments to obtain data was 4 with triplicate samples for the oxygen atmosphere incubations and 7 or greater with triplicate samples for the nitrogen atmosphere incubations.**
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Table 2. Effect of Inspired Oxygen Concentration on the Levels of Chlorodifluoroethylene (CF₂CHCl) and Chlorotrifluoroethylene (CF₂CH₂Cl) in Tail Blood of Rats after Exposure to Halothane

<table>
<thead>
<tr>
<th>Inspired Atmosphere Oxygen Concentration (Per Cent)</th>
<th>CF₂CHCl* (nmol/g Blood) Hours Post Anesthesia</th>
<th>CF₂CH₂Cl* (nmol/g Blood) Hours Post Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>0.11 ± 0.01 0.07 ± 0.00 0.05 ± 0.004</td>
<td>0.44 ± 0.02 0.06 ± 0.01 0.04 ± 0.00</td>
</tr>
<tr>
<td>21</td>
<td>0.12 ± 0.01 0.07 ± 0.00 0.05 ± 0.008</td>
<td>0.57 ± 0.05 0.20 ± 0.04 0.04 ± 0.00</td>
</tr>
<tr>
<td>14</td>
<td>0.57 ± 0.14 0.08 ± 0.01 0.07 ± 0.002</td>
<td>2.18 ± 0.59 0.16 ± 0.03 0.05 ± 0.00</td>
</tr>
</tbody>
</table>

* Data represent mean ± SE for 4 animals/group. Phenobarbital-induced rats were exposed to 1 per cent halothane for 2 h at the designated oxygen concentration. Blood was obtained from the tail vein of rats at the end of anesthesia or at 3 or 24 h post-anesthesia.

The headspace procedure also permitted the measurement of both volatile metabolites in liver tissue after exposure to halothane. The highest levels were obtained in phenobarbital-treated animals exposed to halothane at 14 per cent O₂ (table 4). Even at an oxygen tension of 21 per cent the phenobarbital-treated animal group had higher levels of the metabolites in their liver than the corresponding controls. The amount of CF₂CHCl and CF₂CH₂Cl in the livers of control rats changed very little when the inspired oxygen was decreased to 14 per cent.

In order to assess the potential hepatotoxicity of these two volatile metabolites, phenobarbital-treated rats were exposed to 3500 ppm of CF₂CHCl or 26,500 ppm of CF₂CH₂Cl for 2 hours at 14 per cent O₂. These CF₂CHCl and CF₂CH₂Cl exposures resulted in tail blood levels of 18.2 ± 5.6 and 243 ± 35 nmol/g blood, respectively, at the end of exposure (table 5). At 24 hours after exposure, no morphologic changes were observed by light microscopic examination, and SGPT levels were unchanged from those of control animals. Exposure to CF₂CHCl led to a 25-fold increase in serum inorganic fluoride concentration (49.4 ± 8.9 vs. 1.9 ± 0.3 nmol/g blood in control animals) at the end of the exposure. The CF₂CH₂Cl exposure did not cause an increase in serum F⁻ nor was any CF₂CHCl produced during the exposure (data not shown).

Table 3. Effect of Phenobarbital Pretreatment on the Venous Blood Levels of Chlorodifluoroethylene (CF₂CHCl), Chlorotrifluoroethylene (CF₂CH₂Cl), and Fluoride Following Halothane Exposure

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>CF₂CHCl (nmol/g blood)</th>
<th>CF₂CH₂Cl (nmol/g blood)</th>
<th>Fluoride (nmol/g blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>4 0.8 ± 0.1 2.8 ± 0.4 5.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenobarbital-treated rats</td>
<td>4 2.2 ± 0.5 6.6 ± 1.0 22.5 ± 4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data represent mean ± SE. Animals were exposed to 1 per cent halothane for 2 h in an atmosphere of 14 per cent O₂–85 per cent N₂. At end of anesthesia blood was obtained from the inferior vena cava.

† Number of separate experiments.

‡ When compared to control P < 0.05.

Discussion

In this study we have determined the critical factors necessary for the production of CF₂CHCl and CF₂CH₂Cl from halothane. We found that the in vivo production of CF₂CHCl, CF₂CH₂Cl, and F⁻ was maximal when the rats had been treated with phenobarbital and were exposed to halothane in an hypoxic atmosphere. Microsomal studies supported the in vivo studies since the production of CF₂CHCl, CF₂CH₂Cl, and F⁻ was enhanced when microsomal cytochrome P-450 levels were elevated and when the incubations were performed under a nitrogen atmosphere.
The microsomal studies emphasize the involvement of cytochrome P-450 in the production of CF₂CHCl, CF₂CH₂Cl, and F⁻ from halothane. There was a high degree of correlation between the cytochrome P-450 content and the production of CF₂CHCl, CF₂CH₂Cl, and F⁻, which were formed in almost stoichiometric amounts. The inhibitory effect of CO and the requirement for NADPH are further proof that cytochrome P-450 catalyzes the reductive metabolism of halothane to these metabolites.

The drug metabolism inhibitor, SKF-525A, did not inhibit the microsomal formation of the volatile metabolites of halothane (table 1). However, SKF-525A is known to promote the reduction of cytochrome P-450 and enhance the reductive metabolism of nitrobenzene and neoprontosil. Therefore, it is not surprising that it did not inhibit the reductive metabolism of halothane. In addition, halothane has a high affinity for binding to cytochrome P-450 under anaerobic conditions, thus it may not be readily displaced by SKF-525A. Thus, from the data reported here and by others, it appears that cytochrome P-450 catalyzes both the oxidative and reductive metabolism of halothane.

The microsomal enzymes were also able to metabolize the CF₂CHCl metabolite. Fluoride was liberated when CF₂CHCl was incubated with microsomes in an oxygen atmosphere. Therefore, CF₂CHCl cannot be viewed as a stable end product of halothane metabolism. Evidence of CF₂CHCl metabolism in vivo was also obtained, since a large increase in serum fluoride levels was observed after inhalation exposure to CF₂CHCl. The CF₂CH₂Cl metabolite appears to be resistant to biotransformation since it was not reductively metabolized to CF₂CHCl or inorganic fluoride by microsomes, nor was it converted to trifluoroacetic acid by microsomal mediated oxidative reactions. The blood of rats exposed to high concentrations of CF₂CH₂Cl did not have elevated amounts of fluoride or any detectable CF₂CHCl. However, CF₂CH₂Cl may be bioactivated to an intermediate that efficiently covalently binds to biological macromolecules thus not yielding any detectable amount of metabolites.

Maiorino et al. reported short half-lives for both CF₂CHCl and CF₂CH₂Cl in the blood of patients exposed to halothane anesthesia. Table 2 demonstrates the short half-lives of CF₂CHCl and CF₂CH₂Cl in rats exposed to halothane under various oxygen concentrations. Blood levels of the CF₂CH₂Cl metabolite always exceeded those of CF₂CHCl by three- to four-fold. Interestingly, the levels of CF₂CHCl and CF₂CH₂Cl present in the liver of rats exposed to halothane under hypoxic conditions were equivalent (table 4). This leads one to speculate whether CF₂CHCl and CF₂CH₂Cl are formed in equal amounts by the liver, but during their removal from the body, CF₂CHCl is further degraded. Since we know CF₂CHCl is readily defluorinated during inhalation exposure and rapidly defluorinated by microsomes in an oxygen atmosphere, this may explain the lower level of CF₂CHCl detected in the blood of halothane exposed rats. Faster elimination of CF₂CHCl via exhalation may also explain the difference in blood levels, but little information relative to blood-gas partitioning is available for either

| Table 4. Effect of Phenobarbital Treatment and Inspired Oxygen Concentration on Liver Levels of Chlorodifluoroethylene (CF₂CHCl) and Chlorotrifluoroethane (CF₂CH₂Cl) at End of Halothane Exposure |
|---|---|---|---|
| | Animal Treatment* | CF₂CHCl (nmol/g) | CF₂CH₂Cl (nmol/g) |
| | | | |
| 21 °O₂ in | Control | 22.1 ± 4.9 | 37.3 ± 4.7 |
| Inspired Air | Phenobarbital | 59.1 ± 6.3$ | 69.4 ± 5.0$ |
| 14 °O₂ in | Control | 30.3 ± 3.1 | 41.8 ± 3.8 |
| Inspired Air | Phenobarbital | 114.4 ± 24.8$ | 139.0 ± 32.4$ |

* Rats were exposed to 1 per cent halothane for 2 h at designated oxygen concentration.
† Data represent mean ± SE for n = 4 per group.
‡ P < 0.05 when compared to corresponding control rats.
§ P < 0.05 when compared to induced rats at 21 per cent °O₂.

| Table 5. Effects of Inhalation Exposure to Chlorodifluoroethylene (CF₂CHCl) or Chlorotrifluoroethane (CF₂CH₂Cl) on Serum Inorganic Fluoride, SGPT, and Liver Morphology |
|---|---|---|---|---|
| Chamber Concentration* | Concentration in Tail Blood (nmol/g blood) | Serum F⁻ (nmol/g blood) | SGPT (L.U.) | Liver Morphological Changes |
| | 0 h | 24 h | 0 h | 24 h | |
| CF₂CHCl (3500 ppm) | 18.2 ± 5.6 | 49.4 ± 8.90$ | 2.13 ± 0.12 | 24 ± 3 | None |
| CF₂CH₂Cl (26500 ppm) | 243.0 ± 35.0 | 2.05 ± 0.40 | 2.45 ± 0.06 | 17 ± 4 | None |
| Control | 1.91 ± 0.30 | 2.11 ± 0.17 | 21 ± 4 | None |

* Data represent mean ± SE for 4 animals per group. Phenobarbital-treated rats were exposed to the designated concentration of CF₂CHCl or CF₂CH₂Cl for 2 h in an atmosphere of 14 per cent °O₂.
† Blood obtained at end of anesthesia.
‡ Obtained at 24 h after end of anesthesia.
§ P < 0.001 when compared to control.
compound. The three- to four-fold difference in peripheral blood concentrations of CF$_2$CHCl and CF$_3$CH$_2$Cl vs. central venous blood indicates that they are rapidly eliminated by expiration. Because of this rapid elimination, serum fluoride is likely to remain the most universal indicator of the degree of reductive biotransformation of halothane.

The possibility that CF$_2$CHCl may not be a stable metabolite is also of importance in interpreting the work of Cousin and his colleagues.\textsuperscript{10,18} They calculated a ratio of the expired CF$_2$CHCl/CF$_2$CHCl following exposure to halothane and found that the lower the ratio, the greater the degree of hepatic damage in rats exposed to halothane. This ratio is of value only if the two metabolic products are relatively stable, but should there be further metabolism of CF$_2$CHCl, the ratio would increase and yield false values. Obviously, further studies are required to clarify the extent of the metabolism of CF$_2$CHCl.

The volatile metabolites produced no discernible liver injury when animals were exposed to them via inhalation under conditions that would lead to halothane hepatotoxicity. Raventos and Lemon\textsuperscript{28} also observed no hepatotoxicity after exposure of rats to CF$_2$CHCl delivered in air. However, Brown \textit{et al.}\textsuperscript{25} reported that CF$_2$CHCl administered directly into the portal vein of rats resulted in extensive coagulation necrosis. The reason for this discrepancy is not known, but may relate to the maximum concentration of CF$_2$CHCl in the liver and the rate at which it was attained.

The gas chromatographic assay used would also detect the only other defluorinated halothane derivative that has been identified, 1,1-difluoro-2-bromo-2-chloroethylene.\textsuperscript{8,11} Since none was detected in our \textit{in vivo} or \textit{in vitro} studies, and none has been reported in the expired air of laboratory animals or humans, this derivative is most likely a decomposition product as previously discussed by Sharp \textit{et al.}\textsuperscript{8} However, it is known that this defluorinated product is extremely reactive. If it is formed, it may rapidly become covalently bound to hepatic macromolecules and thus not be detectable as a volatile metabolite.\textsuperscript{26}

It is likely that CF$_2$CHCl and CF$_3$CH$_2$Cl are associated with the reactive intermediates that are formed during the reductive biotransformation of halothane.\textsuperscript{9} This is an important consideration, since increased levels of CF$_2$CHCl and CF$_3$CH$_2$Cl would indicate enhanced metabolism of halothane along its reductive, hepatotoxic route. Recent studies in our laboratory have shown that the hydrogen atom of halothane is retained on both volatile metabolites.\textsuperscript{27} In addition, covalent binding studies with H-halothane have shown that H-containing reactive intermediates are formed and bound to microsomal lipids and protein, only under conditions known to promote reductive metabolism.\textsuperscript{28} Thus, elevated blood or expired air levels of CF$_2$CHCl and CF$_3$CH$_2$Cl would be indicative of enhanced reductive metabolism of halothane.

Measurement of the volatile metabolites could offer a means for rapidly detecting an unusually high degree of reductive biotransformation of halothane. Knowledge of such an event may permit rational therapy to lessen the development of liver injury. For example, administration of certain sulfhydryl containing compounds within six hours after end of halothane anesthesia, resulted in a striking reduction in halothane induced liver injury in the rat hypoxic model.\textsuperscript{19} As our understanding of the mechanism(s) of halothane-induced liver injury increases, such therapy may be useful in clinical anesthesia. At present early markers of potential liver injury are needed. For halothane, monitoring these reductive metabolites may provide early evidence of enhanced biotransformation along its reductive, hepatotoxic pathway.

References