Anesthesiology
50:2-8, 1979

Volatile Metabolites and Decomposition Products of Halothane In Man

J. Howard Sharp, Ph.D., James R. Trudell, Ph.D.,† Ellis N. Cohen, M.D.‡*

The presence of two volatile halothane metabolites, 2-chloro-1,1,1-trifluoroethane (CF_3CH_2Cl) and 2-chloro-1,1-difluoroethylene (CF_2CHCl), and a metabolite-decomposition product, 2-bromo-2-chloro-1,1-difluoroethylene (CF_2CBrCl), were identified by gas chromatography–mass spectrometry in exhaled gases of 16 patients anesthetized with halothane in nonrebreathing, semiclosed and totally closed anesthesia circuits. No significant differences in concentrations of CF_3CH_2Cl and CF_2CHCl were found relative to the anesthesia circuits used. CF_2CBrCl could not be identified in the expired gases of patients anesthetized with a nonrebreathing circuit (Bain), but was present in gases recovered from both semiclosed and totally closed circuits. Under totally closed-circuit rebreathing conditions, the concentration of CF_2CBrCl increased to 4–5 ppm, indicating significant breakdown of halothane by the soda lime. Possible pathways for formation of the two metabolites and the metabolite–decomposition product are presented, as well as clinical implications of these findings. (Key words: Anesthetics, volatile, halothane: metabolites; decomposition products; soda lime interaction; Biotransformation (drug), fluorometabolites; 2-Bromo-2-chloro-1,1-difluoroethylene; 2-Chloro-1,1-difluoroethylene; 2-Chloro-1,1,1-trifluoroethane.)

ALTHOUGH halothane was designed as the prototype of stable hydrocarbon halogenated anesthetics,[1,2] biodegradation of this anesthetic was established within several years of its introduction.[3,4] The liver was shown to be the primary site of halothane metabolism; significant amounts of nonvolatile metabolites are concentrated within this organ.[5] With time, however, these concentrations decrease as the nonvolatile metabolites are slowly excreted by the kidneys,[6] ultimately accounting for 12–24.8 per cent of the absorbed anesthetic.[7,8]

Elimination of smaller amounts of volatile metabolites occur via the lungs. Studies in the rat with intraperitoneally injected ^{14}C-labeled halothane indicated recovery of 1.0–1.2 per cent of the injected dose as $^{14}CO_2$ within 24 hours, providing evidence for complete dehalogenation of the anesthetic.[3] Subsequent studies in heart transplant donor subjects with intravenously administered ^{14}C-labeled halothane yielded 0.4 per cent recovery of $^{14}CO_2$ within six hours.[9] Recently, two additional volatile metabolites of halothane have been demonstrated in the rabbit following inhalational and intraperitoneal administration of this anesthetic.[10] These metabolites, identified as 2-chloro-1,1-difluoroethylene (CF_2CHCl) and 2-chloro-1,1,1-trifluoroethane (CF_3CH_2Cl), attained peak concentrations one hour after anesthesia, reaching levels of 3.9 and 46 ppm, respectively.

The present study of 16 patients has provided identification and quantification of three volatile metabolites or decomposition products of halothane following clinical administration of this anesthetic in closed, semiclosed, and nonrebreathing anesthesia circuits. In these experiments special attention was paid to the influence of carbon dioxide absorption systems upon the decomposition of halothane.

Methods

2-Chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethylene were obtained from PCR Research Chemicals Inc.§ and shown to be more than 99 per cent pure by gas chromatography. 2-Bromo-2-chloro-1,1-difluoroethylene (CF_2CBrCl) was obtained in impure form from the same source and subsequently purified by preparative gas chromatography on a Carbowax 20M column. Analytic gas chromatography was performed with a Varian 2100 gas chromatograph on 2 m × 2 mm i.d. glass columns packed with Carbowax 400 on Porasil C or with Chromasorb 102. Column temperatures were maintained at 55 C and 135 C, respectively.¶ An advantage of the Carbowax 400 Porasil C column for mass spectrometry is that water, which interferes in the mass spectral analysis, is retained on the column during analysis and can be released later by heating. Attempts to remove the water prior to sample injection by use of magnesium sulfate drying tubes[11] proved unsatisfactory due to marked adsorption of the volatile components of the analyte. Combined gas chromatography–mass spectrometry was performed on a Varian 2100 gas chromatograph interfaced through a Finnigan jet separator to a Varian CH-7 mass spectrometer. Mass spectra were measured at 20 eV with source and inlet temperatures of 175 C.

* Lecturer.
† Associate Professor of Chemistry in Anesthesia.
‡ Professor of Anesthesia.
Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305 (Drs. Trudell and Cohen); Department of Chemistry, The New South Wales Institute of Technology, N.S.W., Australia, 2007 (Dr. Sharp). Accepted for publication March 25, 1978. Supported by the National Institute for Occupational Safety and Health, Grant OH 00622-01.
Address reprint requests to Dr. Cohen.

§ PCR Research Chemicals Inc., Gainesville, Fla. 32602.
¶ These columns were selected because halothane was found to decompose to a small extent to CF_3CH_2Cl on Carbowax 20M.

Breath samples from 13 patients were obtained within 5 minutes of the termination of anesthesia by passing a sampling catheter through the disconnected endotracheal tube. These patients had been anesthetized for one to three hours with 0.5–1.5 per cent halothane,** supplemented with various concentrations of nitrous oxide (2.0–3.5 l) and oxygen (2.0–3.5 l). Six of these patients had been anesthetized employing a nonrebreathing Bain circuit (100 ml/kg), and seven, with a high-flow (4–6 l/min) semiclosed soda-lime absorption circuit.

Gas samples from three additional patients were obtained from the rebreathing circuit during anesthesia, within 60 minutes of halothane induction. Anesthesia of two patients was maintained with a semiclosed soda-lime absorption circuit (see above). One of these patients was subsequently changed at the end of an hour to a totally closed-circuit anesthesia technique. In the study of this patient, and an additional patient maintained on closed circuit, halothane concentration was monitored at 0.5–1.0 per cent by ultraviolet analyzer, oxygen by a Clark electrode, end-tidal carbon dioxide by infrared analyzer; nitrous oxide was calculated from the difference.

Because the concentrations of metabolite present were low, it proved necessary first to concentrate the volatile components of the analyte by condensation in a refrigerated 5-ml stainless steel (⅛ inch o.d.) sampling loop filled with glass beads 1 mm in diameter. Gas samples were injected at 50 ml/min through the refrigerated loop attached to a Varian six-port sampling valve, which allowed the condensate to be injected into the carrier gas stream of the gas chromatograph. Using standard mixtures of the volatile components in air, it was found that CF_2CHCl (boiling point −18 C) was trapped with only 15 per cent efficiency at −65 C (isopropanol/dry ice bath). CF_3CH_2Cl (boiling point 6 C) was trapped with 60–70 per cent efficiency under similar conditions. Under more extreme conditions using liquid nitrogen (−196 C), trapping efficiency improved, but oxygen was condensed. Subsequent immersion of the sampling loop in an isopropanol/dry ice bath followed by a 30-second flush of helium (flow 20 ml/min) allowed the liquid oxygen to be flushed away without loss or decomposition of the other, less volatile, components. Under these conditions, the trapping efficiencies for both CF_2CHCl and CF_3CH_2Cl were 60–70 per cent, and those for the other compounds of interest ranged from 75 per cent for CF_2CBrCl (boiling point 42 C) to 80–85 per cent for halothane (boiling point 50 C). Corrections for trapping efficiencies were applied to final calculations of metabolite and halothane concentrations. Large amounts of N_2O, CO_2, and water were also trapped, but these caused little problem in gas chromatography with flame ionization detection.

Results

A typical gas chromatogram on Carbowax 400 Porasil C is shown in figure 1. The peaks at 1.4 and 4.7 minutes correspond in retention times to CF_2CHCl and CF_3CH_2Cl, and were identified as such by mass spectrometry. Mass spectra for these compounds have been described.[10] Although the peak at 3.0 minutes corresponds in retention time to CF_2CBrCl, analysis by gas chromatography–mass spectrometry of samples taken from the patient 5 minutes after anesthesia indicated this peak to be nonhalogenated. It therefore probably represents normal body metabolite(s).

In analyzing gas samples from the patients, it became apparent that those collected from a rebreathing system contained CF_2CBrCl, whereas those collected from a nonrebreathing system, or from the patient 5 minutes after termination of anesthesia, did not. We therefore decided to investigate the possible interaction between soda lime and halothane.[12,13] Samples were collected from the endotracheal tubes in two patients while still connected to a Bain nonrebreathing circuit or to a soda-lime rebreathing circuit. The analyses for CF_2CBrCl were carried out by gas chromatography on a Chromasorb 102 column (fig. 2). On this column at 135 C, CF_2CBrCl has a retention time of 4.8 minutes. A small stable amount of an unidentified nonhalogenated breath metabolite was observed at the same retention time. Background levels of this normal metabolite were established initially for these patients on the Bain nonrebreathing system, before switching to a rebreathing circuit, or from breath samples with a rebreathing circuit before halothane administration was begun. These levels were then subtracted from total peak area to obtain the concentrations of CF_2CBrCl.

As can be seen in table 1, passage of halothane through soda lime in a semiclosed anesthesia circuit (flow 5 l/min) results in production of 0.002–0.005 per cent of CF_2CBrCl relative to the 1 per cent expired halothane concentration. Passage of halothane through soda lime in a totally closed circuit (flow 500 ml/min) in a study of two patients caused production of significantly greater levels of CF_2CBrCl (0.04–0.05 per cent). The CF_2CBrCl rapidly disappeared from the patient's breath within minutes after disconnection from the anesthetic circuit.

** Gas chromatographic analysis of commercial halothane (Ayerst Lot #1GLT) indicated the purity of this preparation. The concentration of no individual contaminant exceeded 0.025 ppm in 1 per cent halothane.

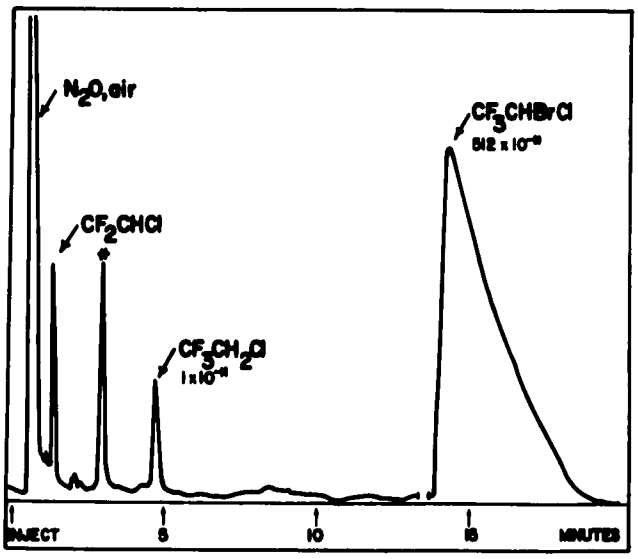

FIG. 1. Gas chromatographic separation of volatile metabolites and decomposition products of halothane. A 50-ml sample from a semiclosed circuit was trapped on a refrigerated loop and separated on Carbowax 400 Porasil C (2 m × 2 mm) at 55 C with a helium flow of 20 ml/min. The baseline break at 13 minutes represents an attenuation in sensitivity of 512-fold (1×10^{-11} to 512×10^{-11}). *Peak represents undefined nonhalogenated breath metabolites.

In order to establish concentrations of CF_2CHCl and CF_3CH_2Cl remaining in the body following halothane anesthesia, samples were collected within 5 minutes from the disconnected endotracheal tubes of 13 unselected patients previously exposed to 0.5–1.0 per cent halothane with a nonrebreathing or a soda-lime rebreathing circuit. These patients varied in age, sex, weight, concentration of anesthetic used, and duration of anesthesia. Analyses were carried out by gas chromatography using a Carbowax 400 Porasil C column (fig. 1). Metabolite concentrations found are shown in table 2. These varied over a relatively wide range. No significant differences in metabolite concentrations were found between the nonrebreathing and semiclosed systems.

Discussion

Although volatile metabolites and decomposition products account for a relatively small fraction of the total biotransformation of the extensively biodegraded halothane molecule, identification of these metabolites is important to gain understanding of the routes of halothane metabolism and in a concern regarding its potential toxicity. The close association between anesthetic biotransformation and anesthetic toxicity has been well established.

Biodegradation of halothane has been shown to proceed along alternate oxidative and reductive pathways. Included among its urinary metabolites are trifluoroacetic acid, N-trifluoroacetyl-ethanolamide, and N-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-cysteine.[14] In addition, free fluoride[6] and free bromide[15] ion are found in the urine. We have previously suggested that the oxidative metabolism of halothane proceeds by means of hydroxylation of the hydrogen on C-2 to form 2-bromo-2-chloro-2 hydroxy-1,1,1-trifluoroethane, which rapidly decomposes to trifluoroacetyl chloride, which then spontaneously hydrolyzes in water to form trifluoroacetic acid.[14] The metabolites described in the present study are dehalogenated by a reductive metabolic process. Pathways for the reductive metabolism of halocarbons have been suggested by Ullrich and Schnabel,[16,17] Van Dyke *et al.*,[18] Brown *et al.*,[19] and the present authors in postulating the existence of a 2-bromo-2-chloro-1,1-difluoroethylene intermediate in the metabolism of halothane.[14]

It is possible to suggest two types of reactions leading to the reductive dehalogenation of halothane. The first is written in analogy to the electrolytic reduction of halothane. Feoktistov demonstrated that halothane was readily reducible, yielding 2-chloro-1,1-difluoroethylene.[20] The facile reduction of halothane has also been demonstrated by Severinghaus *et al.*,[21] and subsequently confirmed by the present authors by voltammetry at a platinum microelectrode (J.H.S.). The

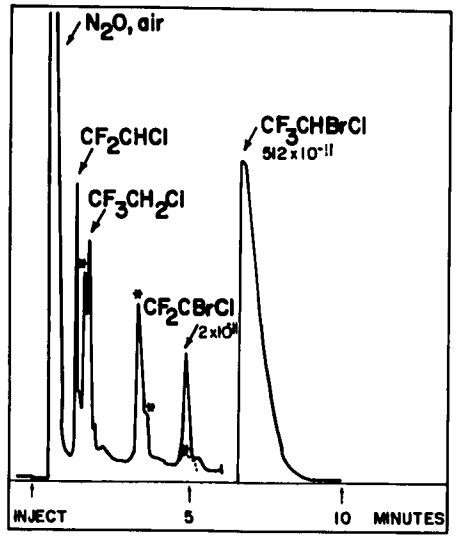

FIG. 2. Gas chromatographic separation of volatile metabolites and decomposition products of halothane. A 50-ml sample from a closed circuit was trapped on a refrigerated loop and separated on Chromasorb 102 (2 m × 2 mm) at 135 C with a helium flow of 10 ml/min. The baseline break at 7 minutes represents an attenuation in sensitivity of 256-fold (2×10^{-11} to 512×10^{-11}). *Peaks represent nonhalogenated breath metabolites.

electrolytic reduction of solutions of halocarbons at metal electrodes has been well studied,[22,23] and also provides a reasonable metabolic pathway. 1,2-dihaloethanes have been shown to accept two electrons and eliminate a halide ion to form an unstable carbanion.[22] This carbanion may then react with a proton from the solvent to form a monohaloethane or eliminate the second halogen to form the unsaturated ethylene.[23] When the ethane has more than one halogen on the same carbon, the carbanion resulting from reduction may lose a second halide ion to form a carbene.[23]

The pathway for two-electron metabolic reduction of halothane is shown in figure 3. Initial transfer of a single electron is followed by loss of bromide ion. Subsequent addition of a second electron to the intermediate (*a*) results in formation of a carbanion (*b*), which may abstract a proton from solvent, a neighboring phospholipid, or an adjacent protein to yield 2-chloro-1,1,1-trifluoroethane (*e*). Alternatively, the carbanion (*b*) may decompose by elimination of a fluoride ion (*c*) to yield 2-chloro-1,1-difluoroethylene (*d*).

The mixed-function oxidase system of the liver, however, is not usually considered to operate by means of a two-electron electrolytic reduction. On the contrary, evidence suggests that cytochrome P-450 transfers electrons by means of a free-radical process. Studies of the dechlorination of carbon tetrachloride[24] and of chloroform,[25] observation of free radicals during metabolism by liver microsomes,[26] the high deuterium isotope effect measured during microsomal hydroxylations,[27,28] and investigations in norbornane systems[29] all substantiate the existence of free-radical intermediates in microsomal metabolism. A proposed scheme whereby halothane is reduced with a single electron, presumably following binding to cytochrome P-450, is shown in figure 4. The reduced intermediate (*a*) eliminates a bromide ion and undergoes further reaction as a free radical (*b*). It may abstract a hydrogen radical to yield 2-chloro-1,1,1-trifluoroethane (*f*). In the process of abstracting a hydrogen radical (for example, from a phospholipid), a free-radical elimination reaction is initiated, which could lead to the diene conjugation observed by Brown *et al.*,[30] or to auto-oxidation of the phospholipid. Alternatively, the chlorotrifluoro radical (*b*) may react by eliminating a fluoride radical (*d*) to form 2-chloro-1,1-difluoroethylene (*e*). The loss of a chloride ion from (*b*) to yield the trifluoroethyl carbene (*c*) has not been demonstrated,[25] but may be the source of the nonvolatile phospholipid and protein binding suggested by Cohen,[31] by Van Dyke *et al.*,[18] and by Hempel and Remmer.[32]

It can be seen that the two-electron pathway in figure 3 and the one-electron radical pathway in figure 4 are similar. Both account for the observed volatile metabolites. The correct pathway depends on whether the cytochrome P-450 system will, in fact, transfer one or two electrons to a halocarbon. At the present time, available experimental data are not sufficient to permit choosing between the possibilities, although a number of implications are associated with the correct mechanism. Abstraction of a hydrogen radical from neighboring phospholipids may initiate major changes in the structure of the phospholipids surrounding the cytochrome P-450. It is also possible that the free radicals, or their carbene product, may destroy components of the drug biotransformation

TABLE 1. CF_2CBrCl in Expired Gases of Patients with Semiclosed and Closed Anesthesia Circuits

	Patient's Sex, Age (Years)	Anesthesia (Min)	CF_2CBrCl* (ppm)
Semiclosed			
Patient 1†	M, 60	60	0.5
Patient 2	F, 60	60	0.2
Closed			
Patient 1	M, 60	60	4.0
Patient 2	M, 62	50	5.0

* Metabolite–decomposition product has been adjusted to correspond to an average halothane concentration of 1.0 per cent (10,000 ppm).

† Patient 1 was transferred from a semiclosed to a closed circuit.

TABLE 2. Concentrations of Volatile Halothane Metabolites with Rebreathing and Nonrebreathing Circuits*

	Patient's Sex, Age (Years)	CF_2CHCl†	CF_2CH_2Cl†
Bain circuit (Nonrebreathing)			
Patient 4	M, 40	0.01	0.09
Patient 5	M, 57	0.03	0.06
Patient 6	F, 18	0.15	0.48
Patient 7	F, 18	0.07	0.56
Patient 8	F, 60	0.07	0.27
Patient 9	M, 50	0.05	0.05
		0.06 ± 0.02	0.25 ± 0.09
Semiclosed circuit			
Patient 10	M, 70	0.13	0.19
Patient 11	M, 2	0.42	1.29
Patient 12	F, 71	0.04	0.07
Patient 13	F, 39	0.02	0.09
Patient 14	M, 3	0.10	0.55
Patient 15	F, 1	0.08	0.20
Patient 16	F, 34	0.16	0.11
		0.14 ± 0.05	0.35 ± 0.15

* 0.5–1.0 per cent halothane administered for 1–3 hours. Samples obtained from the disconnected endotracheal tube within 5 minutes of anesthetic termination.

† Concentration in ppm (±SE).

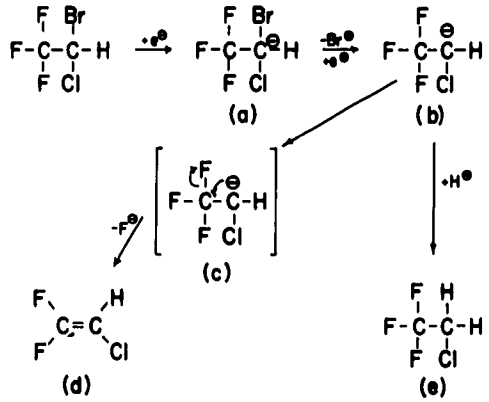

FIG. 3. Two-electron reduction of halothane. An electron is transferred to halothane (*a*), presumably after binding to a reduced cytochrome P-450. A bromide ion is lost, and a second electron is accepted to form a carbanion (*b*). The carbanion eliminates a fluoride ion (*c*) to yield 2-chloro-1,1-difluoroethylene (*d*). The carbanion abstracts a proton from a nearby protein, phospholipid, or solvent to yield 2-chloro-1,1-trifluoroethane (*e*).

system by initiating radical reactions within it or by direct binding to cytochrome P-450. Substances that initiate free-radical reactions have also been shown to be potent carcinogens.[33] It is therefore very important to ascertain whether such radical species are produced by reductive dehalogenation and, if so, to develop methods to block the reductive pathway or provide suitable free-radical scavengers in the body when such a mechanism obtains.

Although it has been suggested that 2-bromo-2-chloro-1,1-difluoroethylene is an intermediary in the metabolism of halothane, resulting in the urinary

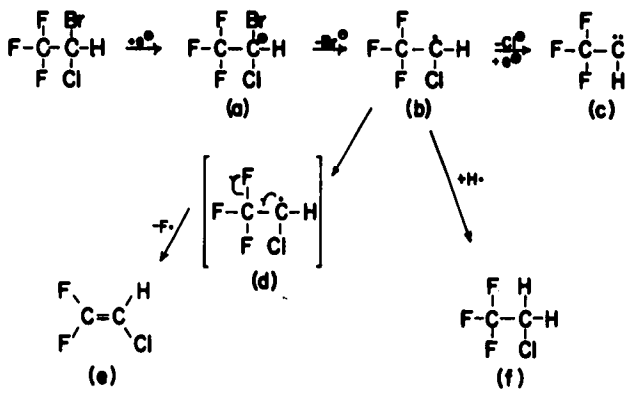

FIG. 4. One-electron reduction of halothane. An electron is transferred to halothane (*a*), presumably after binding to a reduced cytochrome P-450. A bromide ion is lost, creating a free radical (*b*). The free radical may eliminate a fluoride radical (*d*) to yield 2-chloro-1,1-difluoroethylene (*e*). The free radical may abstract a hydrogen radical from a nearby protein, phospholipid, or solvent to yield 2-chloro-1,1,1-trifluoroethane (*f*). The free radical may decompose further to yield a trifluoroethyl carbene (*c*).

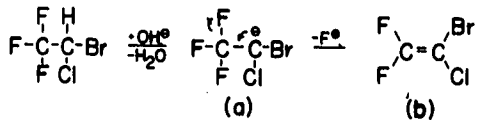

FIG. 5. Base-catalyzed decomposition of halothane. The base abstracts a proton from halothane to yield the carbanion (*a*), which eliminates a fluoride ion to yield 2-bromo-2-chloro-1,1-difluoroethylene (*b*). (This reaction, suggested by Hine,[34] has been confirmed in our laboratory.[14])

metabolite, N - acetyl - S - (2 - bromo - 2 - chloro - 1,1 - difluoroethyl)-cysteine, we did not observe bromochlorodifluoroethylene in the expired gases of patients anesthetized with a nonrebreathing circuit. We suggest that its absence may be associated with a rapid reaction of the metabolite with cysteine in the liver, so that little is returned to the blood for recirculation to the lung and exhalation. In support of this concept, the metabolite–decomposition product is undetectable in expired gases within 5 minutes of disconnection of the patient from the rebreathing circuit. In contrast, exhalation of the CF_3CH_2Cl and CF_2CHCl metabolites continues.

We attribute appearance of the 2-bromo-2-chloro-1,1-difluoroethylene in the expired gases of patients anesthetized with a semiclosed anesthesia circuit to a reaction of the halothane with soda lime. This reaction has been documented by Raventos and Lemon[12] and by Morio *et al.*[13] A suggested mechanism for formation of 2-bromo-2-chloro-1,1-difluoroethylene from halothane in the presence of moist, warm soda lime is shown in figure 5. This scheme is written by analogy to the work on dehalogenation of haloalkanes in aqueous base solution described by Hine *et al.*[34] The basic soda lime abstracts a proton from halothane to yield a carbanion (*a*), which decomposes by elimination of a fluoride ion to yield 2-bromo-2-chloro-1,1-difluoroethylene directly.

The clinical implications associated with presence of the two metabolites and the decomposition product of halothane remain partially defined. CF_3CH_2Cl has been shown to produce convulsions in mice at anesthetic concentration. Its AC_{50} in rats is 43,000 ppm, and its LC_{50} is 150,000 ppm.[12] The levels observed in man were generally less than 1 ppm, well below toxic levels. Recent experiments have shown hepatotoxicity of CF_3CH_2Cl in rats following direct injection of this compound into the hepatic circulation.[20]

Formation of CF_2CHCl and CF_3CH_2Cl as metabolites of halothane has been demonstrated in the rabbit.[10] The present study confirms their presence in man. In rabbits exposed to 1.5 per cent halothane for three hours, both CF_3CH_2Cl and CF_2CHCl remain at

maximal concentrations for one to three hours after termination of anesthesia. Although peak levels of CF_2CHCl in the rabbit reached 3.9 ppm, the concentrations attained in man appear to be lower, *i.e.*, significantly less than 1 ppm. Toxicity studies in rats exposed for seven hours to 5,000–10,000 ppm CF_2CHCl indicate severe degenerative and congestive changes in the kidneys, although no death occurred.[35] It thus seems unlikely that this halothane metabolite would reach toxic concentrations in man.

Greater concern exists in the presence of the metabolite–decomposition product, CF_2BrCl. This compound is very toxic to mice. Animals exposed to 1,000–5,000 ppm die within 48 hours. The LC_{50} of CF_2CBrCl calculated from these experiments approximates 250 ppm.[13] The organs most affected are the kidneys, which show atrophy and degeneration of the convoluted tubules. Although we were unable to detect this compound in the exhaled gases of patients given halothane in a nonrebreathing circuit, the presence of a CF_2CBrCl cysteine conjugate has been demonstrated in the urine of heart-transplant donor subjects.[14] There is thus indirect evidence for formation of this compound as a halothane metabolite in man. Of particular concern is simultaneous generation of the material through an interaction of halothane with soda lime. Experiments with model rebreathing systems indicate that 0.02 per cent of the halothane is transformed into CF_2CBrCl within four hours.[12] Other model system experiments indicate similar concentration.[13] It has been suggested that addition of potassium permanganate to the soda lime may prevent the accumulation of CF_2CBrCl, although this has not been evaluated clinically.** Our clinical studies suggest that significant build-up of this metabolite–decomposition product in man may occur when halothane is administered in a totally closed anesthesia circuit. As indicated in table 1, concentrations of CF_2CBrCl range from 4 to 5 ppm at the end of an hour with an inspired halothane concentration of 1.0 per cent. Although such levels of CF_2CBrCl are significantly less than the determined LC_{50} of 250 ppm in mice, these findings do raise concern regarding the use of closed-circuit halothane administration. Not only is the lethal concentration of CF_2CBrCl in man unknown, but the LC_{01} and the level at which nonlethal damage occurs have not been established. Extended periods of anesthesia and hyperthermia may accentuate any problem that may exist. It is indeed fortunate that with semiclosed administrations (total gas flow 5 l/min), levels of CF_2CBrCl appear to stabilize at less than 1 ppm.

The authors thank Dr. Henry N. Edmunds for the purified samples of 2-bromo-2-chloro-1,1-difluoroethylene and Dr. Charles E. Whitcher for providing much of the clinical material. They are additionally grateful to these individuals for helpful advice and discussion.

References

1. Raventos J: The action of Fluothane: A new volatile anaesthetic. Br J Pharmacol 11:394–410, 1956
2. Duncan WAM, Raventos J: The pharmacokinetics of halothane (Fluothane) anesthesia. Br J Anaesth 31:302–314, 1959
3. Van Dyke RA, Chenoweth MB, Van Poznak A: Metabolism of volatile anesthetics. I. Conversion *in-vivo* of several anesthetics to $^{14}CO_2$ and chloride. Biochem Pharmacol 13: 1239–1247, 1964
4. Van Dyke RA: Metabolism of volatile anesthetics. III. Induction of microsomal dechlorinating and ether-cleaving enzymes. J Pharmacol Exp Ther 154:364–369, 1966
5. Cohen EN, Hood N: Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. III. Halothane. ANESTHESIOLOGY 31:553–559, 1969
6. Rehder K, Forbes J, Alter H, et al: Halothane biotransformation in man: A quantitative study. ANESTHESIOLOGY 28: 711–715, 1967
7. Stier A: Trifluoroacetic acid as metabolite of halothane. Biochem Pharmacol 13:1544, 1964
8. Cascorbi HF, Blake DA, Helrich M: Differences in biotransformation of halothane in man. ANESTHESIOLOGY 32: 119–123, 1970
9. Cohen EN, Van Dyke RA: Metabolism of Volatile Anesthetics. Menlo Park, Calif., Addison-Wesley Publishing Co., 1977
10. Mukai S, Morio M, Fujii K, et al: Volatile metabolites of halothane in the rabbit. ANESTHESIOLOGY 47:248–251, 1977
11. Russell JW, Shadoff LA: The sampling and determination of halocarbons in ambient air using concentration on porous polymer. J Chromatogr 134:375–384, 1977
12. Raventos J, Lemon PG: The impurities in Fluothane: Their biological properties. Br J Anaesth 37:716–737, 1965
13. Morio M, Fujii K, Mukai S, et al: Decomposition of halothane by soda lime and the metabolites of halothane in expired gases. Abstracts, Sixth World Congress of Anaesthesiology, Mexico City, 1976. Excerpta Medica International Congress Series no. 387
14. Cohen EN, Trudell JR, Edmunds HN, et al: Urinary metabolites of halothane in man. ANESTHESIOLOGY 43:392–401, 1975
15. Stier A, Alter H, Hessler H, et al: Urinary excretion of bromide in halothane anesthesia. Anesth Analg (Cleve) 43:723–728, 1964
16. Ullrich V, Schnabel KH: Formation and ligand binding of the fluorenyl carbanion by hepatic cytochrome P-450. Arch Biochem Biophys 159:240–248, 1973
17. Ullrich V, Schnabel KH: Formation and binding of carbanions by cytochrome P-450 of liver microsomes. Drug Metab Dispos 1:176–182, 1973
18. Van Dyke RA, Gandolfi AJ: Anaerobic release of fluoride from halothane, relationship to the binding of halothane

** Topham JC, Imperial Chemical Industries, Ltd., Alderly Park, England, personal communication.

metabolites to hepatic cellular constituents. Drug Metab Dispos 4:40–44, 1976

19. Brown BB, Sipes IG, Baker RK: Halothane hepatotoxicity and the reduced derivative, 1,1,1-trifluoro-2-chloroethane. Environ Health Perspect (in press)
20. Feoktistov LG, Tomilov AP, Goldin MM: Conjugated electrochemical cleavage of halogen. Izv Akad Nauk SSSR, Ser Khim 7:1352, 1963
21. Severinghaus JM, Weiskopf RB, Nishimura M, et al: Oxygen electrode errors due to polarographic reduction of halothane. J Appl Physiol 31:640–642, 1971
22. Fry AJ: Synthetic Organic Electrochemistry. New York, Harper and Row, 1972, pp 170–179
23. Tomilov AP, Mairanozskii SG, Fioshin MY, et al: The Electrochemistry of Organic Compounds. New York, Halsted Press, 1972, pp 281–292
24. Slater TF: Biochemical pathology in microtime. Panminerva Med 18:381–390, 1976
25. Wolf CF, Mansuy D, Nastainczyk W, et al: The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450. Mol Pharmacol 13:698–705, 1977
26. Stier A, Reitz I, Sackmann E: Radical accumulation in liver microsomal membranes during biotransformation of aromatic amines and nitro compounds. Naunyn-Schmiedeberg's Arch Pharmacol 274:189–192, 1972
27. Hjelmeland LM, Aronow L, Trudell JR: Intramolecular determination of substituent effects in hydroxylations catalyzed by cytochrome P-450. Mol Pharmacol 13:634–639, 1977
28. Hjelmeland LM, Aronow L, Trudell JR: Intramolecular determination of primary kinetic isotope effects in hydroxylations catalyzed by cytochrome P-450. Biochem Biophys Res Commun 76:541–549, 1977
29. Groves JT, McClusky RE, White RE, et al: Hydroxylation by highly purified liver microsomal cytochrome P-450. Evidence for a radical intermediate. Biochem Biophys Res Commun 81:154–160, 1978
30. Brown BR: Hepatic microsomal lipoperoxidation and inhalation anesthetics: A biochemical and morphologic study in the rat. ANESTHESIOLOGY 36:458–465, 1972
31. Cohen EN: Metabolism of the volatile anesthetics. ANESTHESIOLOGY 35:193–202, 1971
32. Hempel V, Remmer H: *In vivo* and *in vitro* studies on irreversible binding of halothane metabolites to protein. Experientia 31:680–681, 1975
33. Slater TF: Free Radical Mechanisms in Tissue Injury. London, Pion Press, 1972
34. Hine J: Physical Organic Chemistry. Chapter 8. New York, McGraw-Hill, 1962
35. Torkelson TR, Kary CD, Chenoweth MB, et al: Single exposure of rate to the vapors of trace substances in methoxyflurane. Toxicol Appl Pharmacol 19:1–9, 1971