Toxicology, 23 (1982) 223—234
Elsevier/North-Holland Scientific Publishers Ltd.

TOXICITY OF ULTRAVIOLET-IRRADIATED HALOTHANE IN MICE

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(Received June 26th, 1981)
(Accepted February 8th, 1982)

SUMMARY

Some operating rooms are equipped with ultraviolet (u.v.)-radiating germicidal lamps which can decompose halocarbons. One such agent is the widely used anesthetic, halothane. To study the toxicity of u.v. decomposed halothane, mice were exposed to anesthetic concentrations (1.3%) of non- and u.v.-irradiated halothane in oxygen for 90 min. Halothane sleeping times increased from 14.3 min to 72.5 min. Microsomal mixed function oxidase activity decreased, as shown by prolonged pentobarbital sleeping times 1 day after exposure to halothane and irradiated halothane (54.6 min and 149.1 min, respectively, as compared to a 34.6-min control). Quantitative and qualitative differences were found in the amount of [^{14}C] pentobarbital metabolites excreted by u.v.-irradiated halothane exposed mice compared to either oxygen or non-irradiated halothane exposed groups. In addition, serum glutamic-oxalacetic transaminase (SGOT) of irradiated halothane-exposed mice increased to 233% of the control values, and serum glutamic-pyruvic transaminase (SGPT) were 377% of control values. No significant changes in SGOT or SGPT occurred in non-irradiated halothane-exposed mice. Hepatic cytochromes *P*-450 and b_5 decreased 20% and 13%, respectively, in animals exposed to irradiated halothane, with no significant change in mice exposed to non-irradiated halothane. Microsomal aminopyrine demethylase activity in irradiated halothane-exposed mice also fell to 74% of the control or non-irradiated group values. Decomposition was approximately 10-fold greater for halothane irradiated in oxygen than in nitrogen. Inorganic bromine and fluorine were present, and 9 compounds were recognized by gas-liquid chro-

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Abbreviations: GLC, gas-liquid chromatography; MFO, mixed-function oxidase; SGOT, glutamic-oxalacetic transaminase; SGPT, glutamic-pyruvic transaminase.

0300-483X/82/0000—0000/$02.75

matography. Debromination and formation of 2,2,2-trifluoroacetyl chloride on irradiation in air are hypothesized to be responsible for the increased toxicity. Studies are in progress to evaluate the toxicity of lower concentrations for longer periods and to identify further the decomposition products.

INTRODUCTION

Toxicity has been associated with the use of halothane as an anesthetic [1] and with the chronic exposure to trace levels of halothane present in the operating room [2,3]. Epidemiological surveys have suggested increased occurrences of spontaneous abortions, congenital abnormalities, cancer, and liver disease among operating room personnel as compared to other medical personnel [2,4–6]. Chronic exposures of laboratory animals to subanesthetic halothane concentrations have corroborated some of these findings [7–10], but the issue of chronic toxicity to man is not resolved [11]. The use of germicidal lamps in operating rooms to minimize airborne sources of wound infection [12] may increase the occupational hazard from the inhalation of trace levels of decomposed halothane by operating room personnel. Halocarbons such as halothane are sensitive to the ultraviolet (u.v.) radiation by these lamps [13]. In the gas phase, the carbon-halogen bonds are cleaved homolytically by u.v. light to form carbon centered radicals [14,15]. These reactive intermediates undergo further reactions with atmospheric oxygen or with themselves to form a variety of products [14]. This report contains the results of our investigations on the toxicity of irradiated and non-irradiated halothane to mice and the identification of some of the halothane photodecomposition products. These investigations were undertaken at greater than occupational exposure concentrations to investigate potential mechanisms to toxicity.

METHODS

Halothane vaporization, irradiation and analysis

Unless otherwise mentioned, oxygen (medical grade) was successively passed at 2 l/min through a Fluotec vaporizer and a 2-inch stainless steel tube in which a germicidal Westinghouse lamp (No. 782L-30) was mounted. The resultant gases were then diluted to 1.3% halothane at a flow rate of 6 l O_2/min through the exposure chamber. For the analysis of halothane photodecomposition products, either oxygen, nitrogen, or air was used for vaporization. Gas samples were removed via a syringe through a septum downstream from the exposure chamber and the irradiation apparatus. Analysis of these samples was performed at 25°C in a Varian 3700 gas chromatograph fitted with a 6-foot column containing 10% SP2100 on Chromosorb G-HP 80/100 mesh, (Supelco, Bellefonte, PA). Chromatographic identification of the decomposition products was made by comparison with the retention time of standards. 1,1-Difluoro-2-bromo-2-chloroethylene

(CF_2=CBrCl), 1,1,1-trifluoroacetyl chloride (CF_3COCl), 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene (CF_3CCl=$CClCF_3$), and 1,1,1-tri-fluoroethanol (CF_3CH_2OH) were obtained from PCR Research Chemicals, Gainesville, FL and were used without further purification. HBr was obtained from Fisher Scientific, Raleigh, NC. The spectra of irradiated halothane and bromine were determined in a Cary 118 spectrophotometer fitted with a 10-cm gas cuvette. Inorganic fluoride was measured with an ion-specific electrode (Orion, Cambridge, MA).

Animals

Male CD1 mice (Charles River Laboratories, Wilmington, MA) 7–10 weeks of age were used in these investigations. The mice were kept in cages containing hardwood bedding and housed in isolation chambers through which filtered and purified air passed. The mice were supplied daily with a stock diet (Purina Lab Bloc) and water ad libitum.

Animal exposures and enzyme studies

Groups of mice were exposed for 90 min in a 20-liter glass chamber to either oxygen or 1.3% non- or u.v.-irradiated halothane in oxygen. Immediately following exposure, the mice were removed from the chamber and the time taken to regain the righting reflex recorded as the halothane wake-up time. Twenty-four hours after exposure, mice were divided into 2 groups. One group was injected with sodium pentobarbital (Abbott Labs, Chicago, IL, 50 mg/kg, i.m.), and the loss of the righting-reflex and the sleeping time were determined. All measurements were carried out with the same time schedule in a 37°C controlled temperature room. Liver microsomes were isolated from the other group of mice as described previously [16] except that 5 mM EDTA was substituted for nicotinamide. Microsomal protein was assayed by the method of Lowry et al. [17]. The microsomes were assayed for aminopyrine demethylase activity [18] and the cytochrome *P*-450 and b_5 [19] contents determined. The lungs of these mice were removed and weighed; the wet to dry weight ratios were recorded after drying under vacuum over KOH pellets. Just prior to sacrifice, blood was collected via tail bleeding and serum was isolated for the determination of serum glutamic-oxalacetic transaminase (SGOT; EC 2.6.1.1) and serum glutamic-pyruvic transaminase (SGPT; EC 2.6.1.2) by a commercial procedure (Sigma, St. Louis, MO).

Metabolism of pentobarbital

In another experiment 3 groups of 6 mice each were exposed to 100% oxygen, or 1% irradiated or non-irradiated halothane. Twenty-four hours later each mouse received 2 μCi of sodium 5-[*ring*-2-^{14}C]ethyl-5-(1-methylbutyl)barbitate (New England Nuclear, Boston, MA) diluted with unlabeled sodium pentobarbital (Abbott Laboratories, Chicago, IL) to a final specific activity of 0.301 μCi/μmol and a final dose of 50 mg/kg. Urine and feces were collected over 24 h and 48 h. The total ^{14}C-radioactivity was deter-

mined by oxygen flask combustion. Urine and feces were acidified and extracted with chloroform. The chloroform extracts were evaporated to a dryness and the residue dissolved in acetonitrile. ^{14}C-Urinary metabolites were separated as previously described [20] except that the elution was modified. Elution was performed isocratically for 6 min with 5% acetonitrile (HPLC Grade, Fisher Scientific, Raleigh, NC) followed by a 3%/min linear increasing gradient to 21% acetonitrile in water. Separations were achieved on a 4 mm × 25 cm Ultrasil-ODS column (Altex, Berkeley, CA). Fractions were collected at a flow rate of 1 ml/min, mixed with Redisolv scintillation solution (Beckman Instruments, Norcross, GA), and counted in a Model LS-100 scintillation counter (Beckman Instruments, Norcross, GA).

Statistical analysis

The Student's *t*-test was used to determine the significance of differences between the means. Unless otherwise stated, all data are reported as the mean ± S.E.

RESULTS

Effect of irradiated 1.3% halothane on anesthesia wake-up times and on pentobarbital metabolism and clearance

Post-anesthesia wake-up times were determined in mice after a 90-min exposure to either non-irradiated or irradiated 1.3% halothane. Exposure to irradiated halothane increased wake-up times approximately 5-fold from 14.3 ± 1.2 min to 72.5 ± 9.4 min. Mice exposed to irradiated halothane experienced some respiratory difficulties characterized by periods of apnea. After awakening, these mice were more lethargic and their food and water consumption decreased when compared to non-irradiated halothane exposed mice.

Sodium pentobarbital (50 mg/kg, i.m.) was administered 24 h after halothane exposure to assess changes in its metabolism and clearance by the hepatic microsomal mixed-function oxidase (MFO) system. Loss of righting reflex occurred at 3.02 ± 0.14 min for mice pre-exposed to oxygen; at 3.03 ± 0.10 min for halothane-exposed mice and at 2.85 ± 0.59 min for irradiated halothane exposed mice. These values were not statistically different from each other. Pentobarbital sleeping times after loss of the righting reflex are shown in Fig. 1. Non-irradiated and irradiated halothane pre-exposure values represent an increase over oxygen control exposure of 1.6- and 4.3-fold, respectively (34.6 ± 2.0 min for oxygen controls vs. 54.6 ± 1.8 min for halothane and 149.1 ± 7.7 min for irradiated halothane-exposed). Under these conditions no deaths were recorded for mice exposed to non-irradiated halothane. However, 1 of 33 mice died during the exposure to irradiated halothane and 7 of the 32 remaining mice died after receiving pentobarbital the next day.

[^{14}C]Pentobarbital was cleared rapidly by all groups (Table I) within 24 h. A small fraction was excreted by the u.v.-irradiated halothane exposed

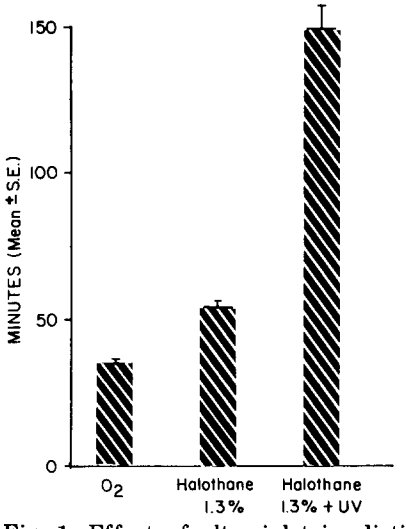

Fig. 1. Effect of ultraviolet irradiation on sodium pentobarbital induced sleeping time. Vertical bars represent S.E. of the means.

group after 24 h. The urinary metabolite profile (Fig. 2) was qualitatively similar in the oxygen and non-irradiated halothane-exposed groups, but not in the irradiated halothane exposed group. The latter excreted a new metabolite (Peak 3A, Fig. 2) not found in the other groups. The chemical structure of this metabolite is unknown.

Effect of irradiated 1.3% halothane on hepatotoxicity

Levels of SGOT and SGPT were determined 24 h following 90-min exposure to oxygen, non-irradiated, or irradiated halothane to assess hepatotoxicity. Table II shows that exposure to non-irradiated halothane resulted in enzyme levels similar to those of controls. Exposure to irradiated halothane resulted in a 2.3-fold elevation of SGOT and a 3.8-fold elevation in SGPT.

TABLE I

EXCRETION OF [^{14}C]PENTOBARBITAL AND ITS METABOLITES BY OXYGEN, HALOTHANE OR U.V.-IRRADIATED HALOTHANE TREATED MICE

Exposure	% of total ^{14}C-radioactivity[a]			
	24 h		48 h	
	Urine	Feces	Urine	Feces
Oxygen	97.91	1.69	0.38	0.02
Halothane	98.94	0.66	0.33	0.07
u.v.-Irradiated halothane	96.18	0.14	3.55	0.14

[a]Pooled values for groups of 6 mice each.

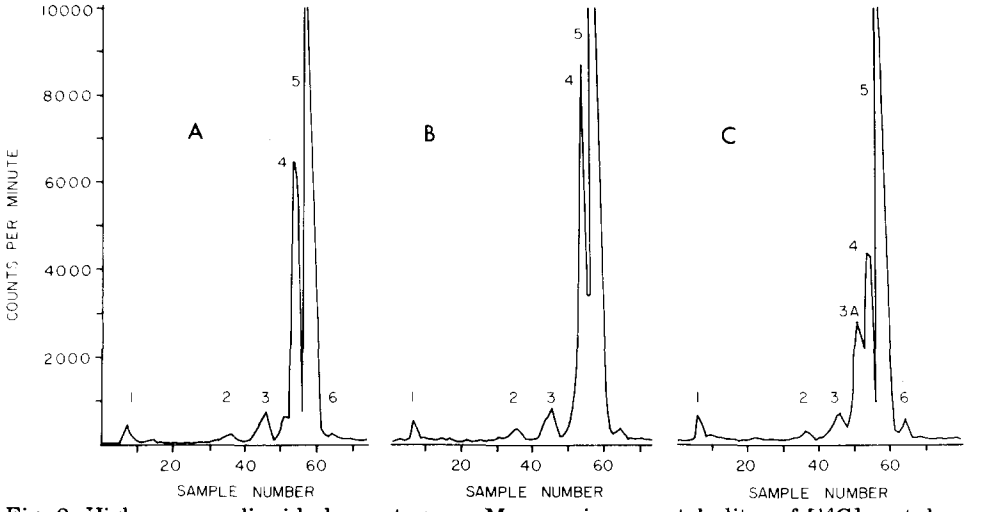

Fig. 2. High pressure liquid chromatogram. Mouse urinary metabolites of [^{14}C]pentobarbital. (A) Metabolites from mice exposed to 100% oxygen alone; (B) those exposed to 100% oxygen containing 1% halothane; and (C) those exposed to 100% oxygen containing 1% halothane following ultraviolet irradiation. Peak 5 is assigned to 5-ethyl-5-(3'-hydroxy-1'-methylbutyl) barbituric acid on the basis of relative retention time. Peaks 1—4 are unknown. Peak 3A appeared only in the urine of mice exposed to u.v.-irradiated halothane (see text for details.)

An examination of liver microsomes from mice exposed to irradiated halothane revealed a significant effect on both MFO activity and components. The effects of irradiated halothane on MFO activity are shown in Table III. No change in aminopyrine *N*-demethylase activity was observed following exposure to non-irradiated halothane. Irradiated halothane exposure resulted in a 26% decrease from the control activity.

The changes in MFO activity were found to correspond to alterations in microsomal cytochrome *P*-450 and b_5 contents (Table IV). Non-irradiated halothane exposure resulted in a slight, but insignificant, decrease in both

TABLE II

EFFECT OF IRRADIATED AND NON-IRRADIATED HALOTHANE ON SERUM TRANSAMINASES OF MICE EXPOSED FOR 90 min TO 1.3% HALOTHANE OR OXYGEN[a]

Exposure	SGOT Sigma-Frankel (units/ml)	N	Control (%)	SGPT Sigma-Frankel (units/ml)	N	Control (%)
Oxygen	52.3 ± 3.0	12	100	25.0 ± 2.4	12	100
Halothane	59.9 ± 6.0	11	115	25.5 ± 2.9	10	102
Halothane + u.v.	121.8 ± 12.2	9[b]	233	94.2 ± 21.5	9[b]	377

[a]Mice were killed 24 h after exposure as described in "Methods".
[b]Satistically different from the oxygen and halothane groups ($P < 0.01$).

TABLE III

EFFECT OF 90 min EXPOSURE TO 1.3% IRRADIATED AND NON-IRRADIATED HALOTHANE ON MURINE HEPATIC MICROSOMAL AMINOPYRINE DEMETHYLASE ACTIVITY[a]

Exposure	Aminopyrine demethylation units	*N*	Control (%)
Oxygen	9.8 ± 0.2	4	100
Halothane	10.0 ± 1.3	5	102
Halothane + u.v.	7.3 ± 0.6[b]	5	74

[a]Mice were killed 24 h after exposure and hepatic microsomes were prepared and assayed as described in "Methods".
[b]Statistically different from the oxygen and halothane values ($P < 0.01$).

hemoproteins. Exposure to irradiated halothane resulted in approximate 20% and 13% losses of cytochromes *P*-450 and b_5, respectively.

Effect of irradiated 1.3% halothane on pulmonary toxicity

Mice experienced respiratory difficulties immediately following exposure to irradiated halothane. A gross examination of the lungs 24 h later revealed hemorrhagic lesions. The lung wet to dry weight ratios were determined to assess the occurrence of edema in all exposure groups. The ratios obtained were 4.4 ± 0.1, 4.6 ± 0.5, and 5.2 ± 0.2 for oxygen, non-irradiated, and irradiated halothane exposures, respectively. Only the latter was significantly different from the control value ($P < 0.01$).

Photodecomposition of halothane by germicidal lamps

Three percent halothane in oxygen, was irradiated for varying periods in

TABLE IV

EFFECT OF IRRADIATED AND NON-IRRADIATED HALOTHANE ON HEPATIC MICROSOMAL HEMOPROTEINS[a]

Exposure	Cytochrome *P*-450			Cytochrome b_5		
	Protein (nmol/mg)	*N*	Control (%)	Protein (nmol/mg)	*N*	Control (%)
Oxygen	1.016 ± 0.030	14	100.0	0.450 + 0.023	14	100.0
Halothane	0.963 ± 0.036	15	94.8	0.434 + 0.016	15	96.4
Halothane + u.v.	0.819 ± 0.044	15[b]	80.6	0.390 + 0.022	15[c]	86.7

[a]Mice were killed 24 h after exposure and hepatic microsomes were prepared and assayed as described in "Methods".
[b]Statistically different from the oxygen and halothane values ($P < 0.01$).
[c]Statistically different from the oxygen and halothane values ($P < 0.05$).

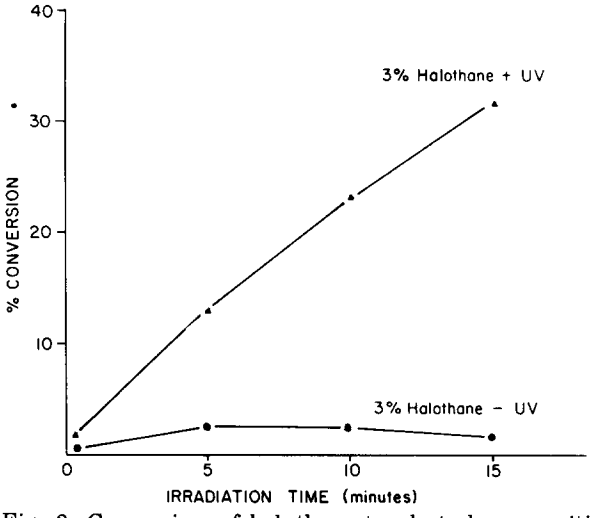

Fig. 3. Conversion of halothane to photodecomposition products by ultraviolet light in 97% oxygen. Conversion was estimated by the decrease in halothane concentration measured by GLC. A small loss of halothane occurred in the absence of irradiation (solid circles).

a static system. Its conversion to products was monitored by a decrease in halothane concentration by gas-liquid chromatography (GLC). Figure 3 shows a linear relationship between irradiation times and conversion of halothane to products.

Because oxygen may interact with free radicals produced during the u.v.-irradiation of halothane, its effect on product distribution was investigated. Sixteen percent halothane was passed through the irradiation apparatus at a flow rate of 40 ml/min, and the decomposition products were analyzed by GLC. Figures 4 and 5 represent chromatograms of halothane irradiated in 100% oxygen and nitrogen, respectively. Both profiles contain peaks 1–3; peaks 4–6, 8, and 9 appear upon irradiation in a nitrogen atmosphere. Peaks 3, 4, and 5 have been identified as CF_3COCl, CF_2CHCl, and CF_3CH_2Cl, respectively. The other peaks have not been identified. Thus far, they have been shown not to correspond to the retention times of either CF_2=$CBrCl$, CF_3CH_2OH, CF_3CCl=$CClCF_3$, or HBr. Under the conditions of the animal exposures only peaks 2 and 3 were significant. We estimate that the mice were exposed to 20 ppm CF_3COCl, peak 3, in these investigations. Although not shown, it was found that chromatographs of halothane irradiated in air and nitrogen were qualitatively similar. (See "Discussion" for a detailed explanation of this apparent ambiguity.) Peak 3, as expected, is significantly increased in air and the relative amounts of peaks 4, 5, 6, 8, and 9 are decreased compared to nitrogen. Photodecomposition, as estimated by the total area of the product peaks, was approximately 10-fold greater for halothane irradiated in air or oxygen than for that irradiated in nitrogen.

Irradiation of 16% halothane in an oxygen atmosphere produces a reddish

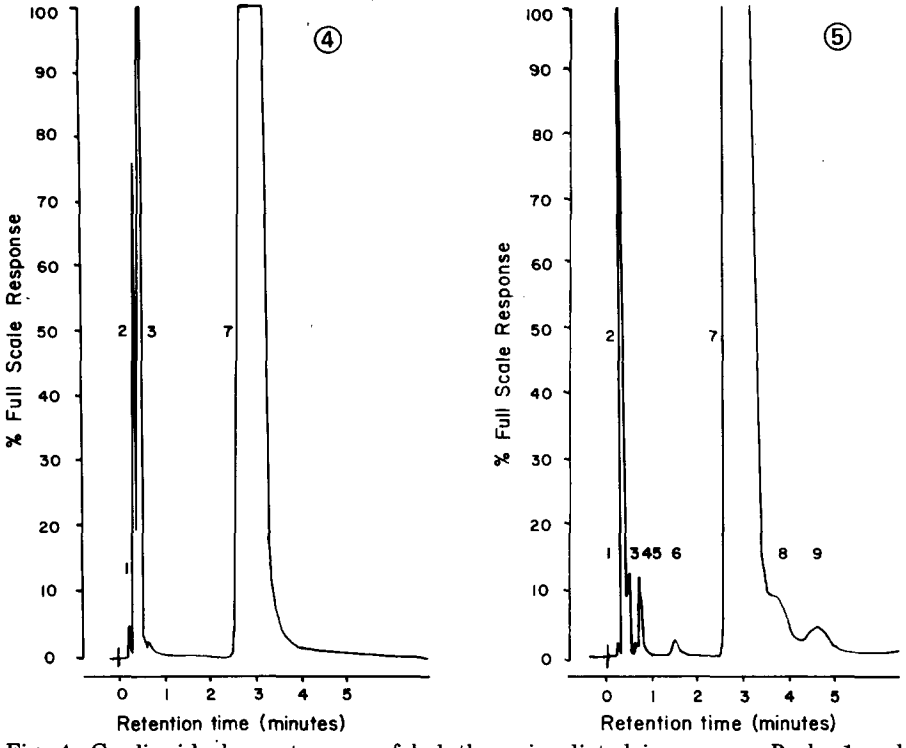

Fig. 4. Gas-liquid chromatogram of halothane irradiated in oxygen. Peaks 1 and 2 are unknown; 3 represents CF_3COCl; and 7 represents halothane. Products were estimated on the basis of retention times of standards.

Fig. 5. Gas-liquid chromatogram of halothane irradiated in nitrogen. Peaks 1, 2, 6, 8 and 9 are unknown; 3 represents CF_3COCl; 4, CF_2=CHCl; 5, CF_3CH_2Cl; and 7, halothane. Products were estimated on the basis of retention times of standards.

brown gas whose visible spectra consist of a broad peak with absorption maxima between 412 nm and 415 nm which correspond to the absorption maximum of bromine gas [21]. This was later confirmed by thiosulfate titration of a solution formed by bubbling the gas through water [21]. Inorganic fluoride was detected in this aqueous solution via an ion specific electrode, suggesting that HF is also a photodecomposition product of halothane.

DISCUSSION

Many xenobiotics are metabolized by the hepatic MFO system to reactive products which subsequently destroy MFO components and precipitate hepatocyte necrosis [22]. Halothane metabolism and its subsequent destruction of hepatic microsomal MFO components have been previously demonstrated [23—26]. The 2- to 4-fold increase in SGOT and SGPT (Table II) observed in mice exposed to irradiated halothane suggest degradation of

halothane to hepatotoxicants. The increased hepatotoxicity of irradiated halothane is further demonstrated by the increased destruction of the microsomal MFO system. Sodium pentobarbital is a substrate for the hepatic MFO system [27], so that prolonged sleeping times after pentobarbital administration can be correlated with altered metabolism. Figure 2 demonstrates an altered metabolism of pentobarbital which is much greater after pre-exposure to irradiated halothane than to non-irradiated halothane. The majority of pentobarbital was cleared from all groups of exposed mice within 24 h (96.18- 98.94%). The altered metabolism of the u.v.-halothane exposed group is shown by the small amount of ^{14}C-radioactivity excreted after 24 h (3.55%). These differences in metabolism are reflected in the urinary metabolite profile found on high-pressure liquid chromatography, where a metabolite (peak 3A, Fig. 2) was found in the urine of mice exposed to u.v.-irradiated halothane but not in those exposed to oxygen or non-irradiated halothane. The observed increase in sleeping time occurred with little change in loss of righting reflex, suggesting that drug distribution is not a major factor. Destruction of the MFO component cytochrome *P*-450 and the closely associated cytochrome b_5 on exposure to irradiated halothane (Table III) also demonstrates the hepatotoxicity of these photodecomposition products. Because several products are formed via the u.v.-irradiation of halothane, the toxic mechanisms involved have yet to be elucidated.

Figure 4 demonstrates the relationship between irradiation times and halothane decomposition. The identities of many of the irradiated halothane by-products are not known. Ultraviolet light emitted by the germicidal lamps used in these investigations is primarily at 253.7 nm [28]. Halothane absorbs at this wavelength [29] and undergoes homolytic cleavage to free radicals [30]. In the gas phase these radicals react to form a number of products

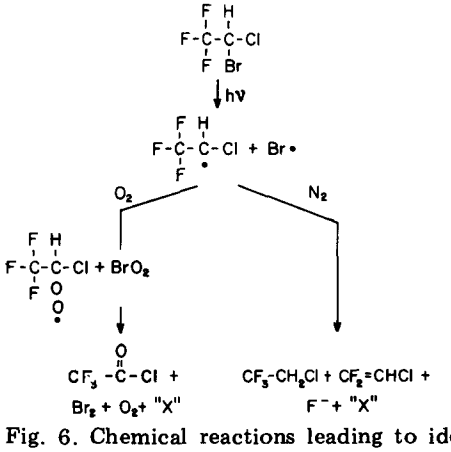

Fig. 6. Chemical reactions leading to identified photodecomposition products of halothane. O_2 refers to the hypothesized reactions in the presence of molecular oxygen, while N_2 refers to those reactions in the absence of molecular oxygen.

(Fig. 6). The relative decomposition was approximately 10-fold greater in the presence of oxygen. Oxygen readily reacts with carbon centered free radicals [30]. Its effect on product formation is demonstrated by Figs. 4 and 5. Bromine gas was identified spectroscopically and chemically as an irradiation by-product in the presence of oxygen. It is likely that it arose from the decomposition of the unstable BrO_2 [21]. For irradiation in an oxygen atmosphere (Fig. 6) only CF_3COCl has been identified as one of the peaks on the GLC profile (Fig. 4). In a nitrogen atmosphere 5 additional peaks are detected (peaks 4–6, 8, and 9; Fig. 4). Peaks 4 and 5 have been identified as $CF_2{=}CHCl$ and CF_3CH_2Cl, respectively.

According to the scheme proposed in Fig. 6, the primary radical $F_3CHCl\cdot$ would react rapidly with molecular oxygen. Even trace amounts of oxygen should be sufficient to react with this $F_3CHCl\cdot$ radical. It is experimentally difficult to remove the traces of oxygen in the nitrogen of the irradiation system, so some of the products arising from the reaction of molecular oxygen and $F_3CHCl\cdot$ were detected in the nitrogen irradiated samples. The rate of reaction of oxygen and similar radicals is so great as to be controlled by the rate of diffusion of the reactants in the gas phase [30]. The concentration of 1,1,1-trifluoroacetyl chloride in samples of halothane irradiated in air was much increased compared to the other components. 1,1,1-Trifluoroacetyl chloride is itself highly reactive and difficult to detect. Depending upon the moisture content of the exposure gas, 1,1,1-trifluoroacetyl chloride could decompose to 1,1,1-trifluoroacetic acid. Both compounds are highly toxic. The effects of these compounds and mixtures of them are being investigated. Unfortunately, mixtures of photodecomposition products occurred despite our efforts to simplify the exposure mixture by the use of oxygen. In the operating room, the exposure conditions are even more complex since other anaesthetic gases (such as nitrous oxide), solvents, and volatile products are also admixed with the halothane photodecomposition products. Our purpose here was to examine some of the toxic properties of an admittedly complex mixture.

In our animal exposures, we estimate from integration of the GLC peaks and decrease in halothane that, under the conditions of exposure, approximately 2% conversion of halothane occurs. This is equivalent to a 90-min exposure of the mice to 260 ppm total decomposition products at which we have observed significant toxicity. Although the conversion of halothane to its decomposition products is low, the toxicity of this mixture is high and worthy of more detailed studies. Of the 260 ppm total by-products formed we estimate that approximately 20 ppm is represented by CF_3COCl. This compound is an acid halide and bears a structural resemblance to the well characterized pulmonary toxicant, phosgene [31]. Like many acid halides, it is susceptible to an electrophilic attack at the carbonyl group by sulfhydryl, hydroxyl, or primary amino groups of many cellular macromolecules. Chronic exposure to this and other decomposition products could, therefore, constitute an occupational hazard. The nature of this risk is under investigation by our laboratory.

ACKNOWLEDGEMENTS

Ms. E. Smolko and M. Eubanks contributed editorial assistance. We wish to thank Professor J.N. Pitts, Jr., who provided expert consultation on the photochemical studies. The work was supported by grant OH 00781 from the National Institute of Occupational Safety and Health. Halothane was generously supplied by Ayerst Laboratories, N.Y.

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