AL-TR-1992-0078

METABOLITE IDENTIFICATION OF HALON REPLACEMENT COMPOUNDS

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JUNE 1992

FINAL REPORT FOR THE PERIOD MARCH 1991 THROUGH MARCH 1992

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AL-TR-1992-0078

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, Nati nal Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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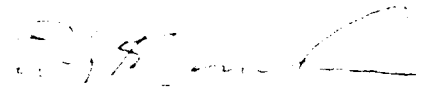

ERIK K. VERMULEN, Colonel, USAF, BSC
Director, Toxicology Division
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REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1992	Final Report, March 1991 – March 1992

4. TITLE AND SUBTITLE

Metabolite Identification of Halon Replacement Compounds

5. FUNDING NUMBERS

Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630214
WU 63021409

6. AUTHOR(S)

W.T. Brashear, M.M. Ketcha, D.L. Pollard, C.S. Godin, H.F. Leahy, P.P. Lu, E.R. Kinkead, R.E. Wolfe

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45431-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AL/OET
Armstrong Laboratory
Wright-Patterson AFB, OH 45433-6573

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

AL-TR 1992-0078

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Halon 1211 is currently being used by the U.S. Air Force as a flight line fire extinguishant. Because of health and environmental concerns over ozone depletion, Halon 1211 must be phased out by the year 2000. Before an interim replacement can be chosen, the toxicity of prospective candidates needs to be evaluated. This study has investigated the metabolism of the Halon replacement candidates perfluorohexane (PFH) and the hydrochlorofluorocarbons (HCFCs): HCFC-123, HCFC-124, and HCFC-142b. Fischer 344 and Sprague-Dawley rats were exposed via inhalation to a 1% atmosphere for 2 h. Tissues were analyzed for volatile metabolites, and urine was analyzed for fluoride and carboxylic acid metabolites. Animals exposed to HCFC-123 or HCFC-124 excreted trifluoroacetic acid in their urine. The presence of trifluoroacetic acid indicates oxidative metabolism of HCFC-123 and HCFC-124. Increased fluoride was found in the urine of rats exposed to HCFC-124. HCFC-142b was metabolized to chlorodifluoroacetic acid, which was found in the urine of exposed rats. Fischer 344 and Sprague-Dawley rats exposed to HCFC-123 had small amounts of HCFC-133a and 2-chloro 1,1-difluoroethylene in liver tissue. The identification of HCFC-133a and 2-chloro-1,1-difluoroethylene following exposure indicates reductive metabolism of HCFC-123. No metabolites of PFH or Halon 1211 were identified in these investigations. Rats exposed to Halon 1211 had slightly elevated bromide levels in their urine compared to control values.

14. SUBJECT TERMS

Gas Chromatography, Gas Chromatography/Mass Spectrometry, Halocarbons, Halon 1211, HCFC-123, HCFC-124, HCFC 142b, Hydrochlorofluorocarbons (HCFCs), Inhalation Exposure, Metabolism, Metabolite Analysis, Perfluorinated Hydrocarbons, Perfluorohexane

15. NUMBER OF PAGES

35

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

PREFACE

This is one of a series of technical reports describing results of experimental laboratory programs conducted in the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc. This document serves as a final report for the study request "Metabolite Identification of Halon Replacements." The research described in this report began in March 1991 and was completed in March 1992. It was performed under Department of the Air Force Contract No. F33615-90-C-0532, Study No. F07. Lt Col James N. McDougal served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Occupational and Environmental Health Directorate, Toxicology Division.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

C	Celsius
CDE	2-Chloro-1,1-difluoroethylene
CDFA	Chlorodifluoroacetic acid
F-344	Fischer 344
g	Gram
GC	Gas chromatography
GC/ECD	Gas chromatography with electron capture detection
GC/MS	Gas chromatography/mass spectrometry
h	Hour
Halon 1211	Bromochlorodifluoromethane
HCFC	Hydrochlorofluorocarbon
HCFC-123	2,2-Dichloro-1,1,1-trifluoroethane
HCFC-124	2-Chloro-1,1,1,2-tetrafluoroethane
HCFC-133a	2-Chloro-1,1,1-trifluoroethane
HCFC-142b	1-Chloro-1,1-difluoroethane
HCl	Hydrochloric acid
kg	Kilogram
L	Liter
m	Meter
M	Moles/liter
mg	Milligram
MHz	Megahertz
min	Minute
mL	Milliliter
mm	Millimeter

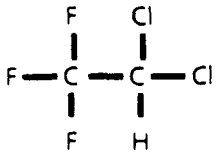
ABBREVIATIONS – Continued

mM	Millimoles/liter
N	Normal solution
NMR	Nuclear magnetic resonance
PAFT	Program for Alternative Fluorocarbon Toxicity Testing
PFH	Perfluorohexane
ppm	Parts per million
s	Second
TISAB	Total ionic strength adjustment buffer
TFA	Trifluoroacetic acid
v	volume
V_{max}	Maximum rate of gas uptake (mg/h/kg)
µg	Microgram
µL	Microliter
µm	Micrometer
µs	Microsecond

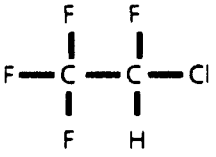
SECTION 1

INTRODUCTION

Environmental concern over the depletion of stratospheric ozone led to the Montreal Protocol of 1987. This international agreement calls for the phaseout of halons by the year 2000. Presently Halon 1211 is being used by the U.S. Air Force as a flight line fire extinguishant. Several candidates have been considered as interim replacements for Halon 1211. Among the candidates are perfluorohexane (PFH) and the hydrochlorofluorocarbons (HCFCs): HCFC-123, HCFC-124, and HCFC-142b. The structures and chemical names of these compounds are shown in Figure 1 and Table 1, respectively



HCFC-123



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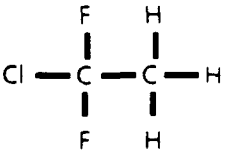
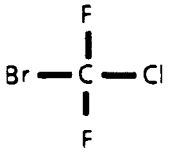
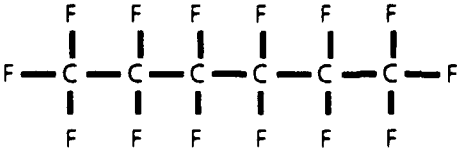


HCFC-142b



Halon 1211



Perfluorohexane

Figure 1. Structures of Halon Replacement Compounds and Halon 1211.

TABLE 1. HALON 1211 AND REPLACEMENT COMPOUNDS

Abbreviation	Chemical Name	CAS #
Halon 1211	Bromochlorodifluoromethane	353-59-3
HCFC-123	2,2-Dichloro-1,1,1-trifluoroethane	306-83-2
HCFC-124	2-Chloro-1,1,1,2-tetrafluoroethane	2837-89-0
HCFC-142b	1-Chloro-1,1-difluoroethane	75-68-3
PFH	Perfluorohexane	86508-42-1

The objective of this study was to identify the toxicologically important metabolites of the Halon replacement compounds. These metabolites were to be compared to the toxicologically important metabolites of Halon 1211.

Ongoing studies of HCFC-123 are being conducted through the sponsorship of the Program for Alternative Fluorocarbon Toxicity Testing (PAFT). A chronic 2-year inhalation study of HCFC-123 is currently in progress where male and female Sprague-Dawley rats are exposed to levels of 0, 300, 1000, and 5000 ppm HCFC-123 for 6 h/day, 5 days/week (Malley et al., 1991). Preliminary results, after 1 year of exposure, indicate that at 300, 1000, and 5000 ppm, male rats had increased hepatic β-oxidation (Malley et al., 1991). Female rats had increased ß-oxidation at 1000 and 5000 ppm. Both males and females had increased liver weights at 5000 ppm (Malley et al., 1991). Urinary fluoride was also increased at all concentrations in both males and females. Metabolism studies of HCFC-123 have shown that trifluoroacetic acid (TFA) is a urinary metabolite that can be detected after a 2-h inhalation exposure to 1% HCFC-123 (Harris et al., 1991). Recent studies have suggested that HCFC-123 is metabolized to a trifluoroacetylchloride intermediate. This can react covalently to form trifluoroacylated liver proteins or may be hydrolyzed to TFA (Harris et al., 1991). The formation of a covalent adduct may be of toxicological significance because the neoantigens formed by exposure to HCFC-123 are immunologically identical to neoantigens from halothane exposure. Halothane has been known to cause a form of hepatitis associated with an immune response against trifluoroacylated liver proteins.

Toxicology studies have been conducted on HCFC-124. These studies have shown that HCFC-124 produced a negative Ames assay result, and did not cause developmental effects in teratology studies performed with male and female Sprague-Dawley rats at exposure levels of 50,000 ppm for 4 weeks (Rusch, 1991). Inhalation studies at 50,000 ppm for 4 weeks demonstrated a transient depressed sensitivity to noise presumably due to the anesthetic action of HCFC-124 (Rusch, 1991). Exposure studies conducted with male Fischer 344 (F-344) rats at 1% HCFC-124 for 2 h have identified TFA and fluoride as urinary metabolites (Olson et al., 1991). Gas-uptake studies have shown that HCFC-124 undergoes less metabolism than HCFC-123 (Hoover et al., 1992). This is due to the presence of an additional chlorine on HCFC-123. The absence of geminal chlorines on HCFC-124 appears to reduce its susceptibility to be metabolized

Studies of the metabolism of HCFC-142b have not been reported. However, toxicological evaluations have found a no-effect-level of 20,000 ppm for general toxicity, genotoxicity, and oncogenicity by inhalation (Seckar et al , 1986). This study exposed groups of 110 Sprague-Dawley-derived CD rats of each sex to whole-body inhalation at levels of 0, 1000, 10,000, and 20,000 ppm (v/v), 6 h/day, 5 days/week, for 104 weeks. After a 90-day inhalation study at 10,000 ppm, rats and dogs did not have increased urinary fluoride levels (Trochimowicz et al., 1977). This study does not

rule out any metabolism of HCFC-142b, but does tend to exclude reductive metabolism because this would involve the cleavage of a carbon–fluorine bond. Oxidative metabolism is more likely for HCFC-142b because there are three hydrogens on one carbon atom. Chlorodifluoroacetic acid is a possible oxidative metabolite of HCFC-142b. HCFC-141b (1,1-dichloro-1-fluoroethane), a hydrochlorofluorocarbon similar to HCFC-142b, has been studied by Harris and Anders (1991). HCFC-141b has been found to undergo dechlorination in male F-344 rats exposed to a 1% atmosphere for 2 h. However, no corresponding metabolites were identified, and covalent adducts of HCFC-141b were not detected. A glucuronide conjugate of 2,2-dichloro-2-fluoroethanol was identified in urine and, at higher exposure levels (4% HCFC-141b, 4 h), 2,2-dichloro-2-fluoroacetic acid was detected in urine. This indicates that the oxidation of 2,2-dichloro-2-fluoroethanol was detectable at higher exposure levels.

Halon 1211 (bromochlorodifluoromethane) may not be appreciably metabolized. The metabolism of trichlorofluoromethane and dichlorodifluoromethane was investigated in beagles via inhalation exposure (Blake and Mergner, 1974). The exposure levels were 1000 to 5000 ppm for trichlorofluoromethane and 8000 to 12,000 ppm for dichlorodifluoromethane. The test animals were exposed to a mixture of radiolabeled and unlabeled halocarbons. The findings of this study indicated that 99% of the halocarbons were expired unchanged. The trace amounts of radioactivity observed in the urine, and the trace amounts of $^{14}CO_2$ observed in expired breath may be due to chemical impurities such as $^{14}CCl_4$ or $^{14}CHCl_3$ in the ^{14}C-labeled halocarbon rather than metabolism (Blake and Mergner, 1974). Metabolism of Halon 1211 would begin with reductive dehalogenation. The carbon–bromine bond would be broken to yield bromide and the corresponding free radical. The analysis of urinary bromide would indicate whether there is any appreciable metabolism of Halon 1211.

Accounts of the metabolism and toxicity of PFH (3M Company, 1990) do not appear in the published literature. However, the manufacturer, 3M Industrial Chemical Product Division of the 3M Company, has supplied basic toxicology data. Tests for acute oral toxicity indicate that PFH is relatively harmless. Eight albino rats were given an oral dose of 34 g/kg with no deaths or remarkable pathological alterations. The Draize test indicated no signs of eye irritation. Tests for irritation on intact and abraded skin found PFH to be minimally irritating. Acute inhalation studies conducted for 1 h at near-saturated vapor concentrations (5260 mg/L in air) did not produce any mortalities or pharmacotoxic signs. No weight loss was reported, and no gross pathologic changes were reported from animal necropsies obtained 14-days postexposure. A 30-day subchronic inhalation study was conducted at near-saturated vapor concentrations. This study, conducted on 26 animals, did not produce any mortalities, abnormal weight patterns, or gross pathologic changes. Some blood chemistry parameters were different from the controls, but were considered to be within a

biologically acceptable range. The results of these toxicology tests indicate that PFH does not have any significant toxicity when administered orally or via inhalation.

In this study, two groups each of male F-344 and Sprague-Dawley rats were exposed to a 1% atmosphere of each test compound for 2 h. Tissue samples from one group were obtained immediately after exposure. The second group was placed in metabolism cages for 24 h. After 24 h, urine, feces, and tissue samples were collected. Samples were analyzed for volatile metabolites by gas chromatography (GC) and GC/mass spectrometry (MS) via headspace sampling with cryofocusing. Urine was analyzed for inorganic fluoride and carboxylic acid metabolites.

Previous investigations of the metabolism and toxicology of halocarbons have used both F-344 and Sprague-Dawley rats. Male and female Sprague-Dawley rats were used for inhalation studies with HCFC-123 (Malley et al., 1991), HCFC-124 (Rusch, 1991), and HCFC-142b (Seckar et al., 1986). However, male F-344 rats were used in 2-h inhalation exposure studies of HCFC-124 (Olson et al., 1991). To account for possible differences in the metabolism of halocarbons between these two strains of rats, both F-344 and Sprague-Dawley rats were used for this inhalation study

SECTION 2

MATERIALS AND METHODS

Test Materials

HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane) and HCFC-124 (2-chloro-1,1,1,2-tetrafluoroethane) were supplied by Allied Signal, Inc. (Morristown, NJ). HCFC-142b (1-chloro-1,1-difluoroethane) was purchased commercially from Matheson Gas Products (Secaucus, NJ) and Halon 1211 (bromochlorodifluoromethane) was purchased from Amerex Corp. (Trussville, AL). Perfluorohexane (FC-72 Fluorinert) was obtained from 3M Corp., Industrial Chemical Products Division (St. Paul, MN).

Animals

Male F-344 and Sprague-Dawley rats were exposed to a 1% atmosphere of the test materials for 2 h. The F-344 rats were 8 weeks old, weighing 150 to 200 g, and the Sprague-Dawley rats were 6 weeks old, weighing 175 to 225 g. Animals were supplied by Charles River Laboratories (Kingston, NY).

Exposure System

The nose-only inhalation exposures were conducted using an 80-port modified Lovelace-type chamber. The unit consisted of animal restraining tubes that mated to a chamber containing the input and exhaust air systems. Input air was directed toward the breathing zone of each port and the exhaust air was removed from the space between the ports. Animals placed in the exposure ports were restrained during the 2-h exposure. Control animals placed in the apparatus were exposed to ambient air.

A valve and flowmeter dilution system generated a 1% atmosphere of the gaseous test materials. These materials were Halon 1211, HCFC-124, and HCFC-142b. Air bubbled through gas washing bottles, followed by dilution, generated an exposure atmosphere of HCFC-123, a single component liquid test material. Perfluorohexane, a multiple component liquid test material, was pumped at an appropriate flow rate and totally evaporated to prevent a distillation effect. Continuous analysis of the chamber exhaust was performed using a Miran 1A CVF infrared monitor (Foxboro Co., South Norwalk, CT).

Reagents

Trifluoroacetic acid, chlorodifluoroacetic acid, and dimethylsulfate were obtained from Aldrich Chemical Co. (Milwaukee, WI). HCFC-133a (2-chloro-1,1,1-trifluoroethane) and 2-chloro-1,1-difluoroethylene (CDE) were obtained from PCR, Inc. (Gainesville, FL). Sulfuric acid, nitric acid, and sodium

hydroxide were obtained from Mallinckrodt, Inc. (St. Louis, MO). Total ionic strength adjustment buffer (TISAB II) for fluoride measurements was supplied by Orion Research, Inc. (Boston, MA). Sodium dodecyl sulfate was obtained from Fisher Scientific (Fair Lawn, NJ).

Equipment

Headspace analysis was performed using a Hewlett-Packard 19395A headspace sampler (Hewlett-Packard, Avondale, PA), which was interfaced to a Tekmar Capillary Interface Model 1000 cryofocusing module (Tekmar, Cincinnati, OH). The cryofocusing module was connected to a Hewlett-Packard 5890 GC interfaced to a Hewlett-Packard 5970 mass selective detector. Chromatographic separation was performed on a PoraPLOT Q capillary column (30 m x 0.32 mm) supplied by Chrompack, Inc. (Raritan, NJ). Headspace analysis was also performed on a Varian 3700 GC equipped with an electron capture detector (ECD) (Varian Associates, Walnut Creek, CA) and a Tekmar Model 7050 automated headspace sampler fitted with a cryofocusing module. The analysis of volatile metabolites was performed on a PoraPLOT Q column (30 m x 0.5 mm). Urinary fluoride was analyzed with an Orion model 96-09 fluoride combination electrode (Orion Research, Inc., Boston, MA). A Haake-Buchler vortex evaporator (Saddlebrook, NJ) was used for heating and vortexing hydrolysis samples and derivatization mixtures.

Statistical Analysis

Urinary fluoride data was analyzed using a two-way analysis of variance. The Bonferroni test for multiple comparisons was used to test for differences between the exposure groups and the controls. Statistical software was provided by BMDP Statistical Software (Los Angeles, CA).

SECTION 3

EXPERIMENTAL

Exposure Methodology

Fischer 344 and Sprague-Dawley rats were exposed to a 1% atmosphere of the test materials for 2 h via nose-only inhalation. Groups of eight animals plus two controls were used for each test material. The two control animals were exposed to ambient air using the same apparatus. Immediately after exposure, 4 test animals and 1 control animal were euthanatized. Samples of liver, kidney, heart, lung, muscle, skin, fat, testes, and blood were quick-frozen in liquid nitrogen. The samples were stored at -20 °C for analysis. The 4 remaining test animals and 1 control animal were put into metabolism cages for 24-h urine and feces collection. During the collection, urine was maintained at 0 °C and feces were maintained below room temperature. After collection, these samples were stored at -20 °C for analysis. After 24 h the 4 test animals and 1 control animal were euthanatized. Samples of liver, kidney, heart, lung, muscle, skin, fat, testes, and blood were quick-frozen in liquid nitrogen. These samples were stored at -20 °C for analysis.

Chemical Analysis

Headspace analysis by GC with ECD was performed on a 0.5-g (or 0.5-mL) sample placed into a 23-mL headspace sample vial. The headspace sampler was heated to 70 °C, and the headspace vapor space was focused for 0.3 min at 0 °C. After focusing, the sample was heated to 100 °C and analyzed chromatographically. The chromatography conditions are shown in Table 2.

TABLE 2. CHROMATOGRAPHY CONDITIONS

	GC/MS	GC/ECD
Initial temperature	50 °C	50 °C
Initial time	0 min	0 min
Heating rate	5 °C/min	5 °C/min
Final temperature	200 °C	200 °C
Carrier gas	Helium	Helium
Flow rate	1.8 mL/min	1.9 mL/min
Make-up gas	None	15 mL/min Ar/CH_4
Injection port temperature	130 °C	150 °C
Detector temperature	225 °C	300 °C
Mass spectrometer transfer line	250 °C	None
Headspace sample loop	1.0 mL	0.10 mL

Headspace analysis by GC/MS was performed on a 0.5-g (or 0.5-mL) sample placed into a 23-mL headspace vial. The headspace sampler for GC/MS analysis was held at 40 °C. The headspace vapor sample was focused for 0.67 min onto a liquid nitrogen cooled capillary. The capillary was then heated and analyzed according to the conditions in Table 2.

Tissues were also analyzed by the headspace method after hydrolysis to promote the release of volatile components. Sample (0.5 g or 0.5 mL) was placed into a 23-mL headspace vial with 0.5 mL of 10 M sodium hydroxide and was vortexed for 3 h at 50 °C. The mixture was neutralized with 0.35 mL of concentrated nitric acid and was vortexed for 1 h at 50 °C. The hydrolysis mixture was allowed to cool and was analyzed by the headspace method.

Fluoride analysis was performed on urine collected 24 h after inhalation exposure. A 2-mL aliquot was obtained from the urine collected from each exposed animal. The sample was centrifuged at 1500 × *g* for 2 min at room temperature. The supernatant was decanted and 100 µL was combined with 100 µL of TISAB II. A millivolt reading was obtained, and the fluoride ion concentration was determined from a standard curve generated from control rat urine.

Urine samples were analyzed for TFA, chlorodifluoroacetic acid (CDFA), and bromide by derivatization to form volatile methyl esters using the method of Maiorino (Maiorino et al., 1980). A sample of urine (100 µL) was pipetted into a 23-mL headspace vial and placed on ice, and 500 µL of cold concentrated sulfuric acid was added. While on ice, 100 µL of dimethyl sulfate derivatizing agent was added, and the vial was sealed. The solution was vortexed at 60 °C for 20 min, allowed to cool, and analyzed by the headspace method with cryofocusing. Quantitative analysis of TFA was performed on samples of 24-h urine from two F-344 and two Sprague-Dawley rats from the HCFC-123 and HCFC-124 exposure groups. Trifluoroacetic acid concentrations were measured using a calibration curve of TFA-spiked urine standards. Quantitative analysis of bromide excreted by F-344 and Sprague-Dawley rats exposed to Halon 1211 was performed using a calibration curve of sodium bromide (NaBr)-spiked urine standards. Chlorodifluoroacetic acid was used as an internal standard. Because of the low concentrations of bromide present in these samples, GC/MS analysis was done using selected ion monitoring. The ion at 94 *m/z* was monitored for bromide, and the ion at 85 *m/z* was monitored for CDFA.

^{19}F NMR Spectrometry

The test materials investigated contain fluorine, which can be detected by radiolabeled fluorine nuclear magnetic resonance (^{19}F NMR) spectrometry. This technique can detect volatile as well as nonvolatile metabolites that contain fluorine. Selected samples of liver, urine, and testes from F-344 and Sprague-Dawley rats exposed to HCFC-123, PFH, and Halon 1211 were analyzed by ^{19}F NMR at the University of Kentucky College of Pharmacy (Lexington, KY). Tissue samples (0.5 g) were

homogenized in a minimum amount of saline. Prior to analysis, 0.5 mL of sample was combined with 0.5 mL of water. Neat urine samples (0.5 mL) were combined with 0.5 mL of water. This 1.0 mL mixture was placed into a 5.0 mm nuclear magnetic resonance (NMR) tube, and a 30-µL aliquot of 25 mM trifluoroethanolamine was used as an external standard. The samples were analyzed on a Varian 300-VXR NMR spectrometer (Varian Associates, Palo Alto, CA) operating at the fluorine resonance frequency of 282.203 MHz. The ^{19}F NMR spectrum of the urine samples and tissue homogenates consisted of 9600 transients. Each transient required a 1.5-s acquisition time with a 9.3-µs radiofrequency pulse (60° flip) for a total of a 4-h acquisition time for each sample.

Microsomal and cytosolic fractions from the liver of a Sprague-Dawley rat exposed to 1% HCFC-123 were analyzed for trifluoroacetylated protein lysine adducts by ^{19}F NMR spectrometry. The subcellular fractions were prepared from a perfused liver according to the procedure of Harris et al., (1991). After a 2-h exposure to HCFC-123, the rat was placed into a metabolism cage for 24 h, then it was sacrificed and the liver was perfused. The liver was then homogenized, and the cytosolic and microsomal fractions were obtained by differential centrifugation. The fractions were dialyzed at 4 °C for 48 h against a pH 7.0, 10 mM phosphate buffer containing 0.1% sodium dodecyl sulfate. The dialyzed fractions were lyophilized and assayed for protein content. The lyophilized cytosolic and microsomal fractions were reconstituted in denterium-labeled water (^2H_2O) to obtain a protein concentration of at least 30 mg/mL and were analyzed by ^{19}F NMR with an acquisition time of 24 h. These samples were analyzed by the University of Kentucky as previously described.

SECTION 4

RESULTS

HCFC-123

The HCFC-123 used for the inhalation exposures was found to contain about 5% HCFC-123a (1,2-dichloro-1,1,2-trifluoroethane). The parent compound was detected in tissue samples collected immediately after exposure in both F-344 and Sprague-Dawley rats. HCFC-123 was detected using either GC or combined GC/MS methods. Hydrolysis of the tissue samples was not necessary to detect the presence of HCFC-123 immediately following exposure. HCFC-123 was not detected in most samples collected 24 h after exposure. However, HCFC-123 was detected in the liver, kidney, muscle, and skin. The results from the analyses for the parent compound are summarized in Table 3.

TABLE 3. ANALYSIS OF PARENT COMPOUND: HCFC-123 EXPOSURE[a]

	F-344		Sprague-Dawley	
	0 h	24 h	0 h	24 h
Liver	+	-	+	+[b]
Kidney	+	-	+	+[b]
Skin	+	+[c]	+	-
Muscle	+	+[c]	+	-
Testes	+	-	+	-
Heart	+	-	+	-
Blood	+	-	+	-
Lung	+	-	+	-
Fat	+	-	+	-
Urine	NA	-	NA	-
Feces	NA	-	NA	-

[a] + = HCFC-123 detected
- = HCFC-123 not detected
NA = sample not available.
[b] Hydrolyzed tissue sample
[c] Detected by GC/ECD only

HCFC-133a (2-chloro-1,1,1-trifluoroethane) was detected in liver and kidney samples from F-344 and Sprague-Dawley rats immediately following exposure to HCFC-123. The total-ion chromatogram of an HCFC-123-exposed liver sample is shown in Figure 2, and the mass spectrum of HCFC-133a is shown in Figure 3. The parent compound had a retention time of 18.6 min, and the HCFC-133a had a retention time of 13.2 min. The mass spectrum shown in Figure 3 matches an authentic standard of HCFC-133a. HCFC-133a was also detected in liver samples that were hydrolyzed

with sodium hydroxide and neutralized with nitric acid. Here, the intensity of the HCFC-133a in relation to the HCFC-123 peak is greater than a headspace sample analyzed without hydrolysis. In a hydrolyzed sample, the HCFC-133a peak area is approximately 9.6% of the HCFC-123 peak area, whereas in a headspace sample, the HCFC-133a peak area is approximately 0.3% of the HCFC-123 peak area. Trace amounts of CDE were found in the livers of F-344 and Sprague-Dawley rats exposed to HCFC-123. The total-ion chromatogram is shown in Figure 4, and the mass spectrum of CDE is shown in Figure 5. This matches an authentic mass spectrum of 2-chloro-1,1-difluoroethylene.

Trifluoroacetic acid was found in urine samples from both F-344 and Sprague-Dawley rats. The total-ion chromatogram of a derivatized urine sample from an F-344 rat is shown in Figure 6, and the mass spectrum of TFA methyl ester is shown in Figure 7. This mass spectrum matches an authentic standard of TFA methyl ester. In a 24-h period, F-344 and Sprague-Dawley rats excreted 18 ± 5 μmoles (n = 4) TFA.

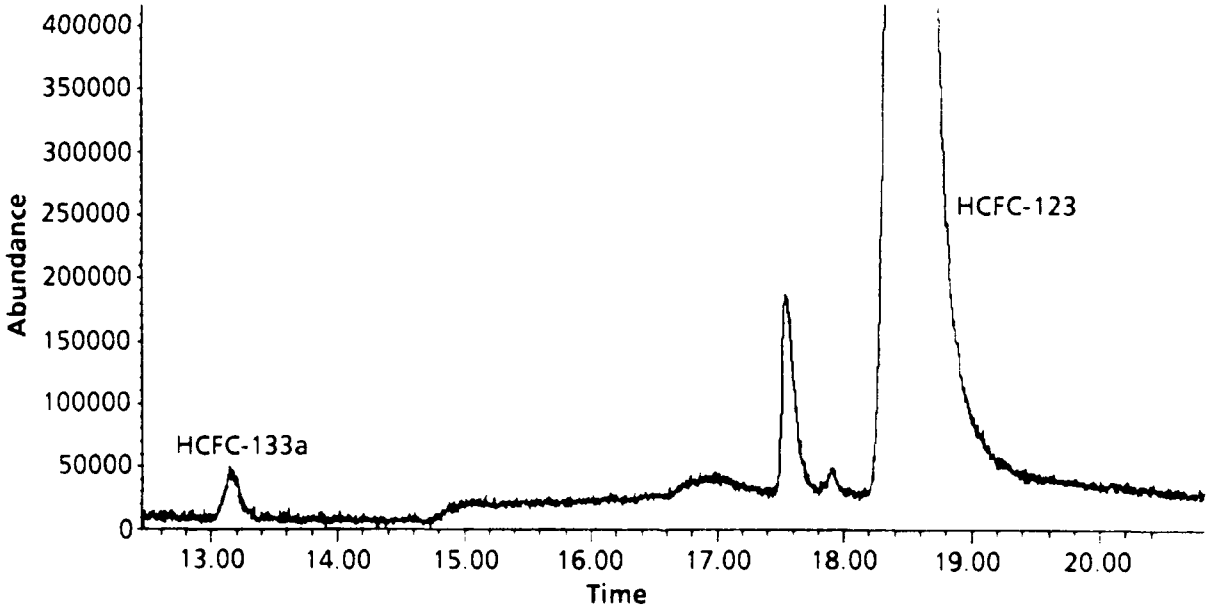


Figure 2. Total-Ion Chromatogram of a Liver Headspace Sample Obtained from an F-344 Rat Exposed to HCFC-123. HCFC-133a has a retention time of 13.2 min and HCFC-123 has a retention time of 18.6 min.

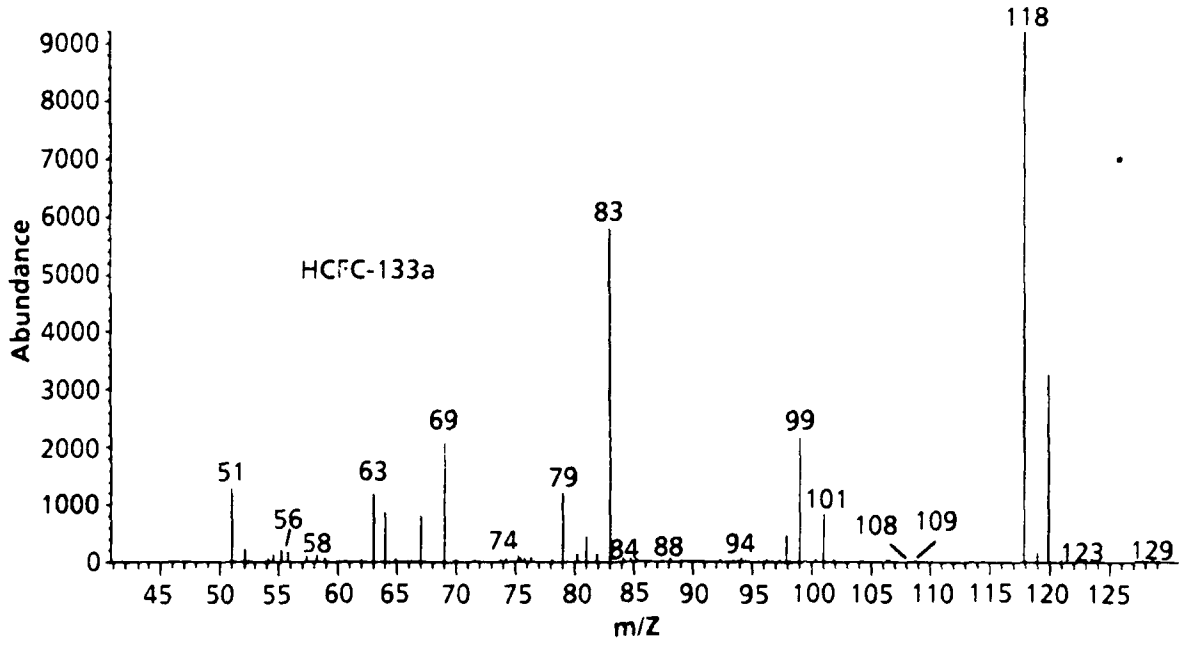


Figure 3. Mass Spectrum of HCFC-133a Obtained from a Liver Headspace Sample of an F-344 Rat Exposed to HCFC-123.

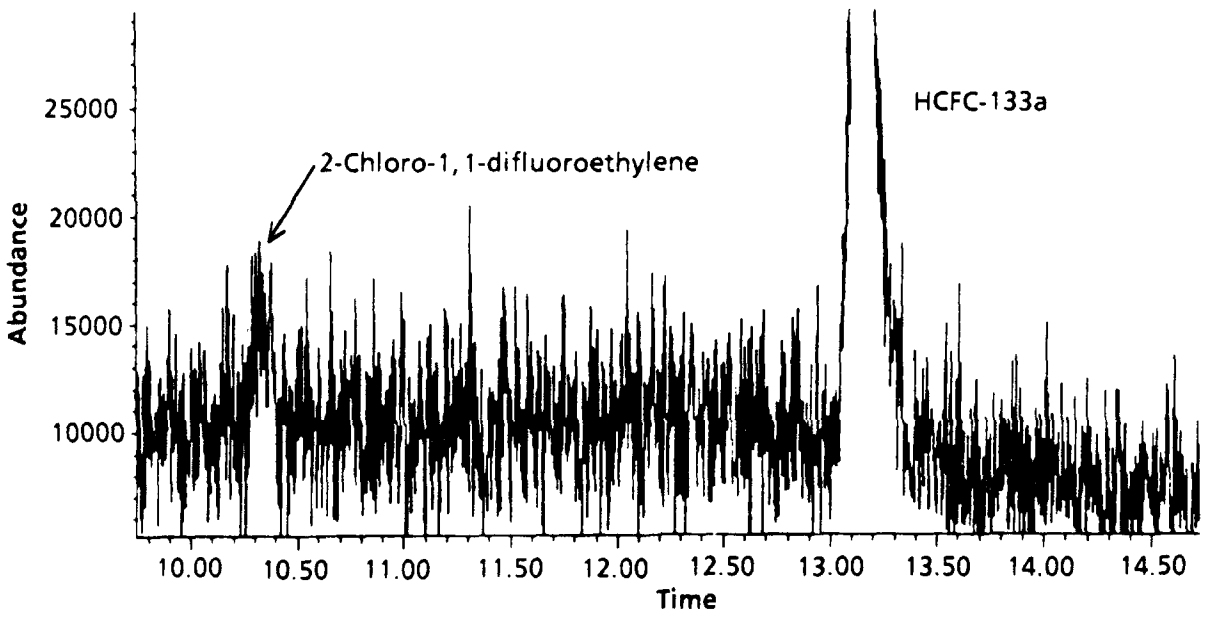


Figure 4. Total-Ion Chromatogram of a Liver Headspace Sample Obtained from an F-344 Rat Exposed to HCFC-123. 2-Chloro-1,1-difluoroethylene has a retention time of 10.3 min; also shown is HCFC-133a with a retention time of 13.2 min.

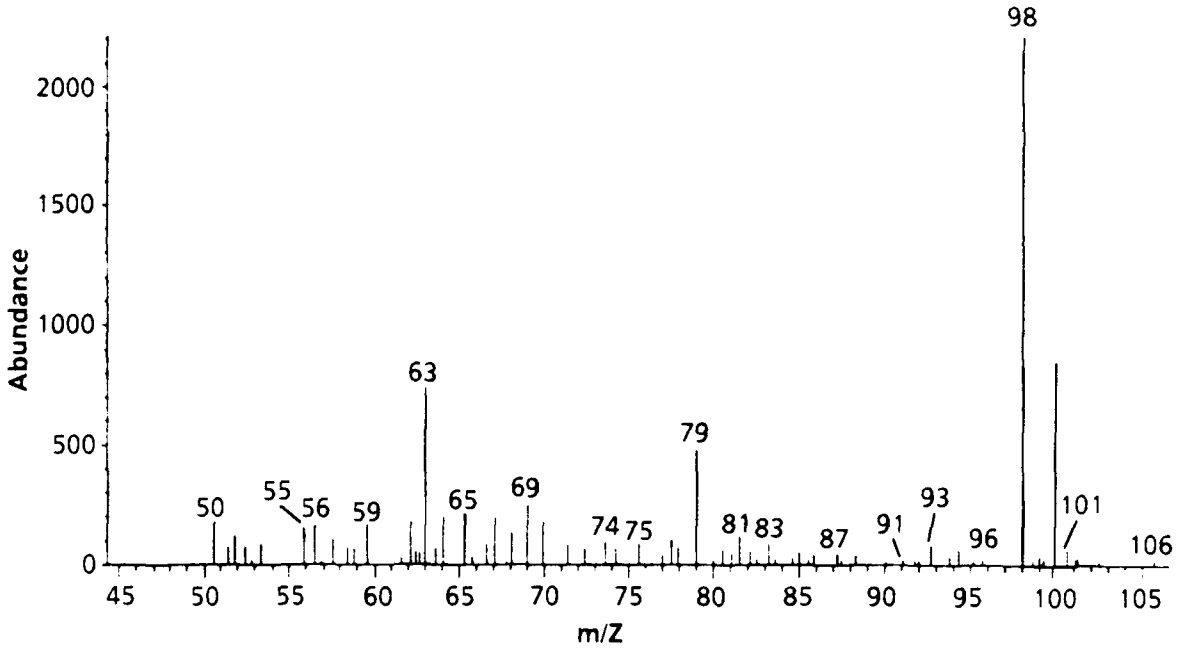


Figure 5. Mass Spectrum of 2-Chloro-1,1-difluoroethylene Obtained from a Liver Headspace Sample of an F-344 Rat Exposed to HCFC-123.

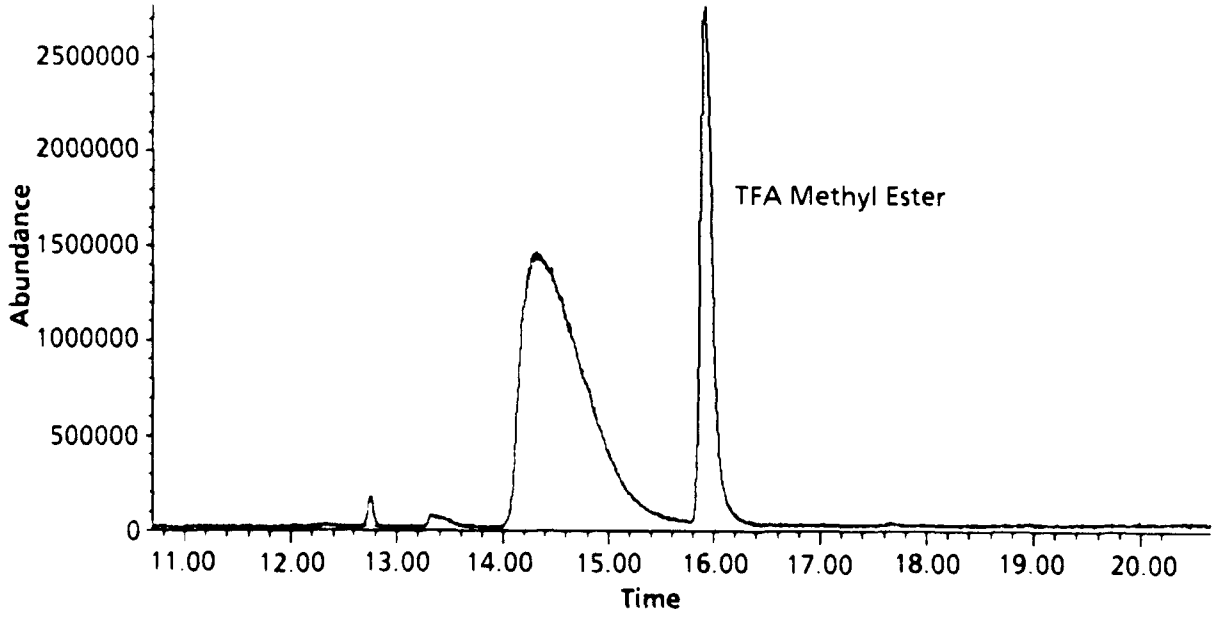


Figure 6. Total-Ion Chromatogram of a Dimethylsulfate-Derived Urine Sample Obtained from an F-344 Rat Exposed to HCFC-123. The methyl ester of TFA has a retention time of 15.9 min.

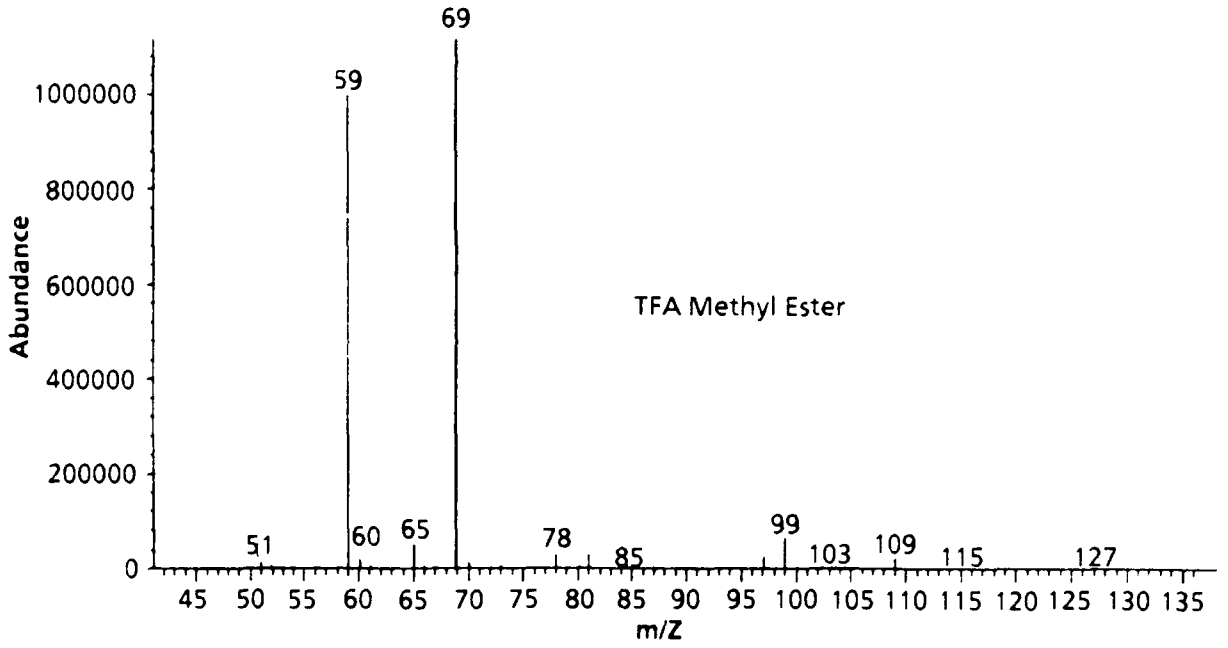


Figure 7. Mass Spectrum of the Methyl Ester of TFA from a Dimethylsulfate-Derived Urine Sample Obtained from an F-344 Rat Exposed to HCFC-123.

HCFC-124

Parent compound (retention time 11.4 min) was detected in tissue samples obtained immediately after exposure to HCFC-124, and was not detected in samples collected 24 h following exposure (including samples analyzed after hydrolysis). These results are summarized in Table 4. There was good agreement between results obtained via GC and GC/MS. No volatile metabolites were detected by the headspace analysis of HCFC-124 samples.

TABLE 4. ANALYSIS OF PARENT COMPOUND: HCFC-124 EXPOSURE[a]

	F-344		Sprague-Dawley	
	0 h	24 h	0 h	24 h
Liver	+	-	+	-
Kidney	+	-	+	-
Skin	+	-	+	-
Muscle	+	-	+	-
Testes	+	-	+	-
Heart	+	-	+	-
Blood	+	-	+	-
Lung	+	-	+	-
Fat	+	-	+	-
Urine	NA	-	NA	-
Feces	NA	-	NA	-

[a] + = HCFC-124 detected
- = HCFC-124 not detected
NA = sample not available

Urine collected from F-344 and Sprague-Dawley rats for 24 h after exposure was derivatized with dimethylsulfate (Maiorino et al., 1980). The volatile methyl ester of TFA was detected in the urine of both F-344 and Sprague-Dawley rats. The mass spectrum of the TFA methyl ester matched the mass spectrum of an authentic standard. In a 24-h period, F-344 and Sprague-Dawley rats excreted 2.1 ± 0.3 μmoles (n = 4) TFA.

HCFC-142b

Parent compound (retention time 11.3 min) was detected in tissue samples obtained immediately after exposure to HCFC-142b, and was not detected in samples collected 24 h following exposure (including samples analyzed after hydrolysis). These results are summarized in Table 5. No volatile metabolites were detected by the headspace analysis of HCFC-142b samples. A urinary carboxylic acid metabolite of HCFC-142b was detected in 24-h urine collected from F-344 and Sprague-Dawley rats. The metabolite was identified as CDFA. A total-ion chromatogram of the volatile methyl ester of CDFA is shown in Figure 8, and the mass spectrum of the CDFA methyl ester is shown in Figure 9. The mass spectrum matched an authentic standard of CDFA methyl ester.

TABLE 5. ANALYSIS OF PARENT COMPOUND: HCFC-142b EXPOSURE[a]

	F-344		Sprague-Dawley	
	0 h	24 h	0 h	24 h
Liver	+[b]	-	+	-
Kidney	+[b]	-	+	-
Skin	+	-	+	-
Muscle	+[b]	-	+	-
Testes	+[b]	-	+	-
Heart	+[b]	-	+[b]	-
Blood	+[b]	-	+[b]	-
Lung	+[b]	-	+[b]	-
Fat	+	-	+	-
Urine	NA	-	NA	-
Feces	NA	-	NA	-

[a] + = HCFC-124 detected
- = HCFC-124 not detected
NA = sample not available
[b] Detected by GC/MS and not detected by GC/ECD

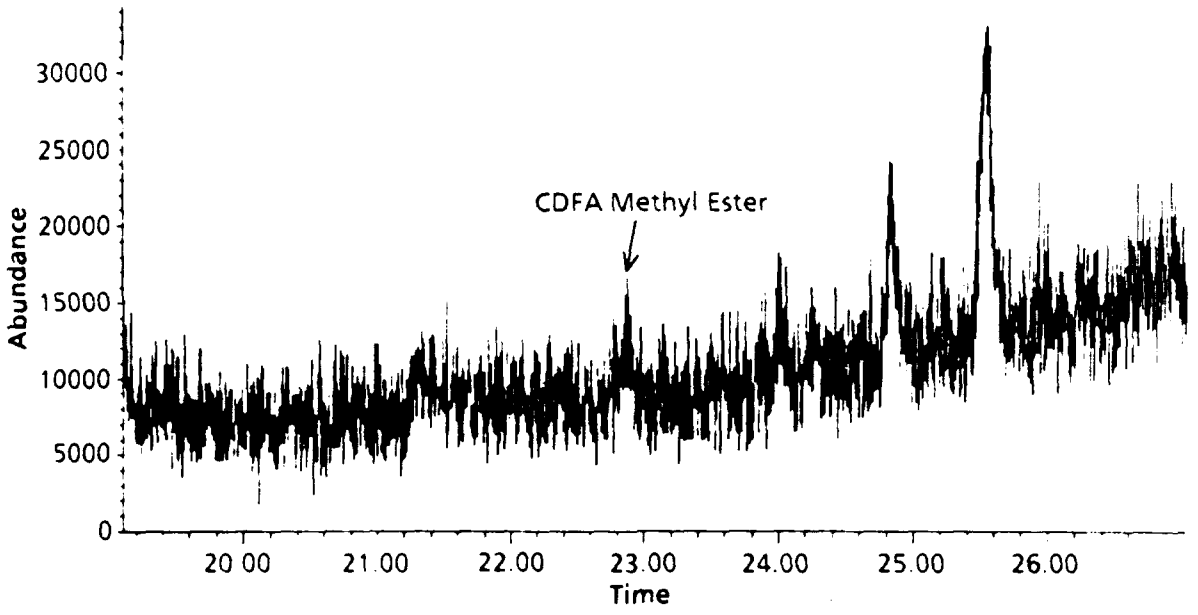


Figure 8. Total-Ion Chromatogram of a Dimethylsulfate-Derived Urine Sample Obtained from an F-344 Rat Exposed to HCFC-142b. The methyl ester of CDFA has a retention time of 22.9 min.

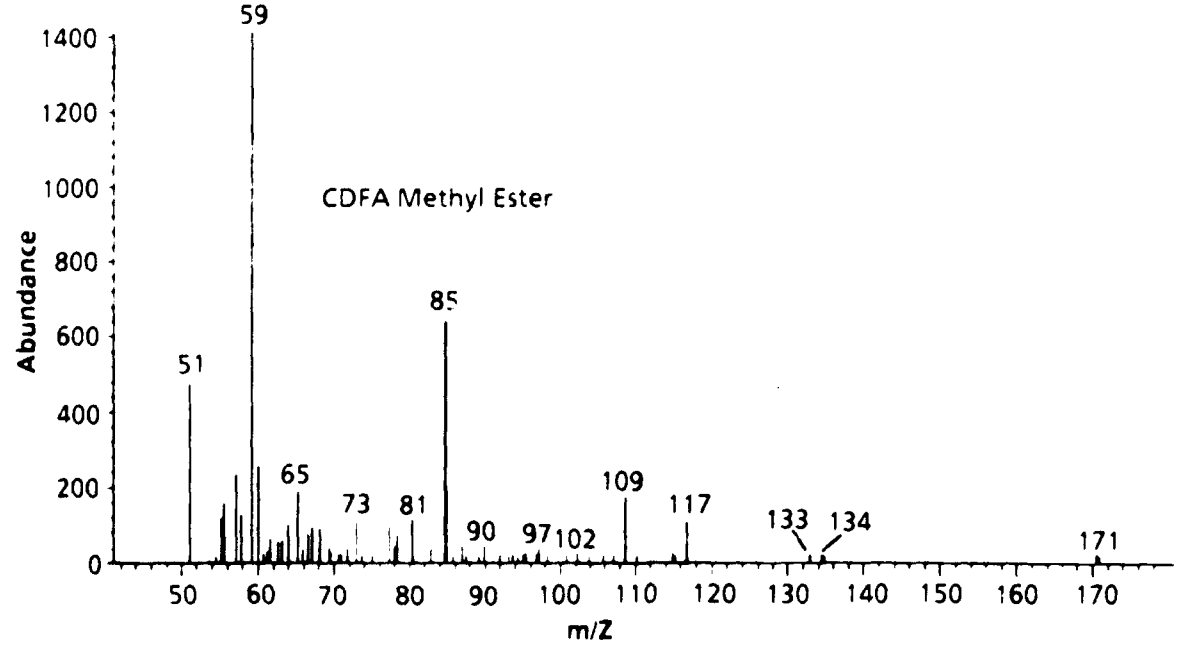


Figure 9. Mass Spectrum of the Methyl Ester of CDFA from a Dimethylsulfate-Derived Urine Sample Obtained from an F-344 Rat Exposed to HCFC-142b.

Perfluorohexane

Metabolites of PFH were not detected in tissue samples, or in the urine of F-344 or Sprague-Dawley rats. Immediately after exposure, parent material was observed in tissues, but 24 h following exposure, PFH was found only in fat. The results are summarized in Table 6.

TABLE 6. ANALYSIS OF PARENT COMPOUND: PERFLUOROHEXANE EXPOSURE[a]

	F-344		Sprague-Dawley	
	0 h	24 h	0 h	24 h
Liver	+	-	+	-
Kidney	+	-	+	-
Skin	+	-	+	-
Muscle	+	-	+	-
Testes	+	-	+	-
Heart	+	-	+	-
Blood	+	-	+	-
Lung	+	-	+	-
Fat	+	+	+	+
Urine	NA	-	NA	-
Feces	NA	-	NA	-

[a] + = PFH detected
- = PFH not detected
NA = sample not available

Halon 1211

Parent compound (retention time 16.4 min) was detected in samples obtained immediately after exposure to Halon 1211 and in some samples collected 24 h after exposure. However, no volatile metabolites of Halon 1211 were detected. These results are summarized in Table 7.

TABLE 7. ANALYSIS OF PARENT COMPOUND: HALON 1211 EXPOSURE[a]

	F-344		Sprague-Dawley	
	0 h	24 h	0 h	24 h
Liver	+	+[b]	+	-
Kidney	+	-	+	-
Skin	+	+[b]	+	+[b]
Muscle	+	+[b]	+	+[b]
Testes	+	+[b]	+	-
Heart	+	-	+	-
Blood	+	-	+	-
Lung	+	-	+	-
Fat	+	+[b]	+	-
Urine	NA	+[b]	NA	+[b]
Feces	NA	-	NA	-

[a] + = HCFC-124 detected
- = HCFC-124 not detected
NA = sample not available
[b] Detected by GC/ECD only

Urine collected from F-344 and Sprague-Dawley rats 24 h after exposure to Halon 1211 was analyzed for bromide. Sprague-Dawley rats were found to have urinary bromide levels higher than control animals ($p < 0.05$). Control rats excreted 0.6 ± 0.1 μmoles ($n = 4$) of bromide in 24 h, whereas exposed rats excreted 1.0 ± 0.2 μmoles ($n = 4$) in 24 h. Urinary bromide from F-344 rats was elevated, but the elevation was not statistically different from the controls ($0.05 < p < 0.10$).

Urinary Fluoride

Following exposure, 24-h urine was collected from 4 F-344 and 4 Sprague-Dawley rats. Each set of 4 animals had 1 control. The fluoride measurements (micrograms per milliliter) were multiplied by the amount collected to obtain the total amount excreted in 24 h. The results are summarized in Table 8.

TABLE 8. URINARY FLUORIDE EXCRETION[a]

	F-344 (μg F^-/24 h)	Sprague-Dawley (μg F^-/24 h)
HCFC-123	1.7 ± 1.3[a]	0.5 ± 0.4
HCFC-124	26.1 ± 4.6	25.9 ± 5.6
HCFC-142b	13.8 ± 2.0	13.5 ± 4.7
Halon 1211	14.5 ± 2.7	13.0 ± 3.5
PFH	4.0 ± 1.0	8.0 ± 5.7
Control[b]	6.3 ± 3.5	9.3 ± 8.1

a Mean ± standard deviation ($n = 4$)

b $n = 5$

Analysis of variance indicated a significant difference in the amounts of fluoride excreted over 24 h among the different test materials and the controls ($p = 0.00001$, $F[5,21] = 36.6$), but not between the two strains of rats. Multiple comparisons demonstrated that the urinary fluoride from the HCFC-124-exposed animals was significantly greater than the controls for both F-344 and Sprague-Dawley rats ($p < 0.01$). The data in Table 8 show that test animals exposed to HCFC-142b and Halon 1211 excreted more fluoride than the control animals, and test animals exposed to HCFC-123 and PFH excreted less fluoride than the control animals. However, these differences were not statistically significant.

^{19}F NMR Spectrometry

The results of the ^{19}F NMR analysis detected TFA in the urine of F-344 and Sprague-Dawley rats exposed to HCFC-123. The TFA fluorine resonance at 4.7 ppm was confirmed by adding an authentic standard of TFA. The addition of TFA increased the intensity of the fluorine resonance at 4.7 ppm, confirming its identity as TFA. Analyses of urine from F-344 and Sprague-Dawley rats exposed to PFH and Halon 1211 did not detect any urinary metabolites. The analysis of HCFC-123-, PFH-, and

Halon 1211-exposed tissue samples did not provide evidence of additional metabolites. Liver cytosolic and microsomal fractions from an HCFC-123-exposed F-344 rat were analyzed for a trifluoroacetylated lysine adduct. The ^{19}F NMR analysis did not detect the presence of a trifluoroacetylated protein adduct after acquiring data on 30-mg/mL protein samples for 24 h.

SECTION 5

DISCUSSION

Studies of exposure of rats to 1% of HCFC-123, HCFC-124, HCFC-142b, PFH, and Halon 1211 indicate that these test materials are taken up by inhalation and are almost completely eliminated after 24 h. Only liver, kidney, muscle, and skin from rats had detectable levels of HCFC-123, and fat had detectable levels of PFH after 24 h. Halon 1211 was found in liver, skin, muscle, fat, testes, and urine after 24 h only by GC/ECD. Electron capture detection is more sensitive towards carbon–bromine bonds than carbon–chlorine or carbon–fluorine bonds. Therefore, the increased sensitivity of GC/ECD may account for the apparent persistence of Halon 1211 in exposed tissue.

Animals exposed to HCFC-123 had TFA in urine but did not have increased levels of urinary fluoride. The amount of fluoride excreted by F-344 and Sprague-Dawley rats was not significantly different from the controls. Previous studies (Malley et al., 1991) have indicated that urinary fluoride is increased in Sprague-Dawley rats exposed to HCFC-123. However, this was a chronic study involving larger numbers of animals (n = 80 per group). Trifluoroacetic acid was detected in the urine of HCFC-123-exposed F-344 and Sprague-Dawley rats by both GC/MS and ^{19}F NMR spectrometry. Urinary TFA is indicative of oxidative metabolism, which is the anticipated mode of metabolism. The ^{19}F NMR analysis did not detect the presence of a trifluoroacetylated lysine adduct in hepatic cytosolic and microsomal fractions obtained from the liver of an HCFC-123-exposed F-344 rat. Other investigators have detected this protein adduct by ^{19}F NMR (Harris et al., 1991) and it is not clear why this was not detected in our experiments. However, it may be possible to detect a trifluoroacetylated liver protein using more sensitive immunological techniques.

The analysis of headspace vapor from HCFC-123 liver and kidney samples have detected the presence of HCFC-133a and CDE. HCFC-133a is a reductive metabolite of HCFC-123. Studies conducted on orally dosed male and female rats (300 mg/kg for 1 year) have associated HCFC-133a exposure with uterine adenocarcinomas and benign interstitial cell neoplasms of the testes (U.S. Environmental Protection Agency, 1990). 2-Chloro-1,1-difluoroethylene is another reductive metabolite of HCFC-123. *In vitro* studies have shown that CDE binds to and inactivates microsomal cytochrome P_{450} (Baker and Bates, 1988). The inactivation of cytochrome P_{450} is believed to occur through an epoxide intermediate (epoxides are known to be reactive towards macromolecules), and the formation of an epoxide-cytochrome P_{450} transient intermediate is consistent with the metabolic end products of CDE: glyoxylic and glycolic acid (Baker et al., 1990). It is possible that the small amount of CDE observed in this study in relation to the amount of HCFC-133a may be due to its high degree of reactivity. Finding CDE and HCFC-133a in the liver of F-344 and Sprague-Dawley rats exposed to HCFC-123 indicates that a reductive pathway participates in the metabolism of HCFC-123. This pathway produces two metabolites that may be toxicologically important. The proposed

metabolism of HCFC-123 is shown in Figure 10. The reductive pathway begins with reductive dehalogenation to produce a radical intermediate that can accept a hydrogen atom from a protein or phospholipid to form HCFC-133a, or lose fluorine to yield CDE. The metabolism of HCFC-123 is identical to the reductive metabolism of halothane ($CF_3CHBrCl$), where HCFC-133a and CDE are the reductive metabolites (Sharp et al., 1979). The oxidative pathway catalyzed by cytochrome $P_{450}IIE1$ produces a dichloro geminal halohydrin, which is unstable, and releases hydrochloric acid (HCl) to form an acylchloride, which is readily hydrolyzed to TFA.

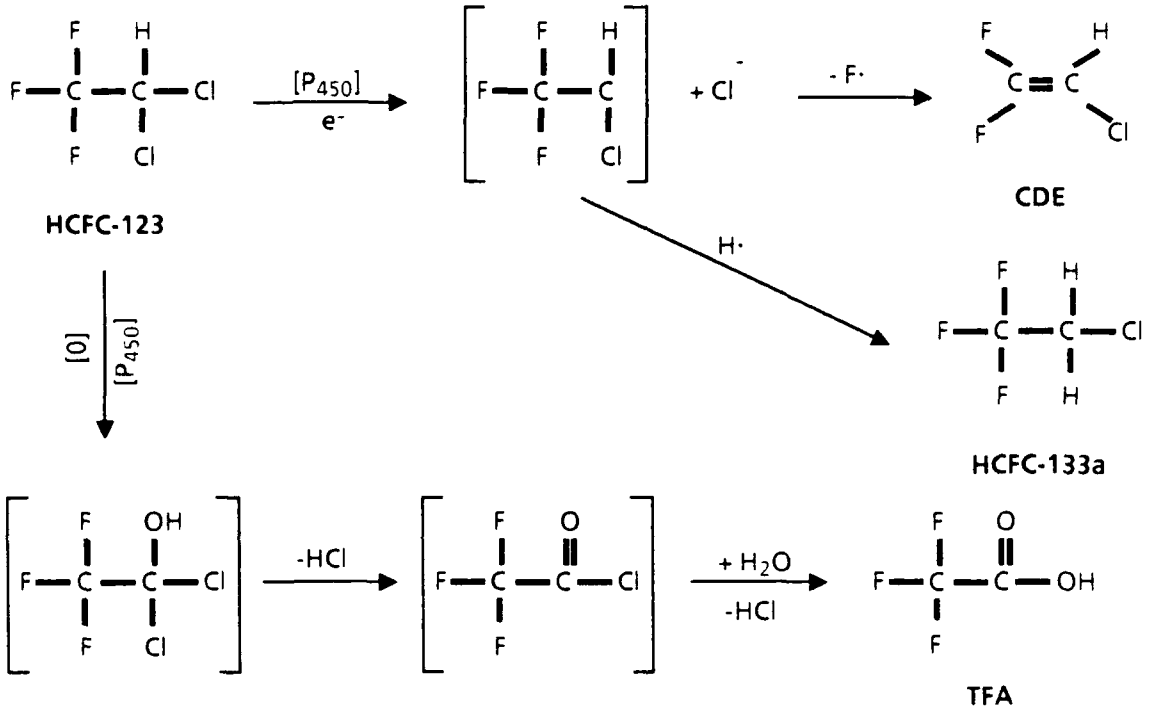


Figure 10. Proposed Metabolic Pathway for HCFC-123 Showing Reductive Metabolism to HCFC-133a and CDE and Oxidative Metabolism to TFA.

Analysis of urine from rats exposed to HCFC-124 found TFA and increased levels of fluoride. This is consistent with previously reported findings (Olson et al., 1991). The presence of urinary fluoride is not indicative of reductive metabolism because oxidation of HCFC-124 to TFA will release fluoride. The oxidative metabolism of HCFC-124 is analogous to the oxidative metabolism of HCFC-123. A geminal halohydrin is formed via oxidation. This releases HCl to form a highly reactive acylfluoride, which is hydrolyzed to TFA. The metabolism of HCFC-124 is shown in Figure 11. Reductive metabolites of HCFC-124 were not observed. These metabolites may be present in concentrations below the limit of detection. The amounts of TFA found in 24-h urine indicate that HCFC-124 is metabolized less than HCFC-123. Gas-uptake studies have also shown that HCFC-124 has a much lower maximum rate of gas uptake (V_{max}) than HCFC-123 (Hoover et al., 1992). The decreased metabolism of HCFC-124 in relation to HCFC-123 may be attributed to the absence of a second

reactive chlorine on the number two carbon making HCFC-124 less likely to undergo reductive dehalogenation or oxidation.

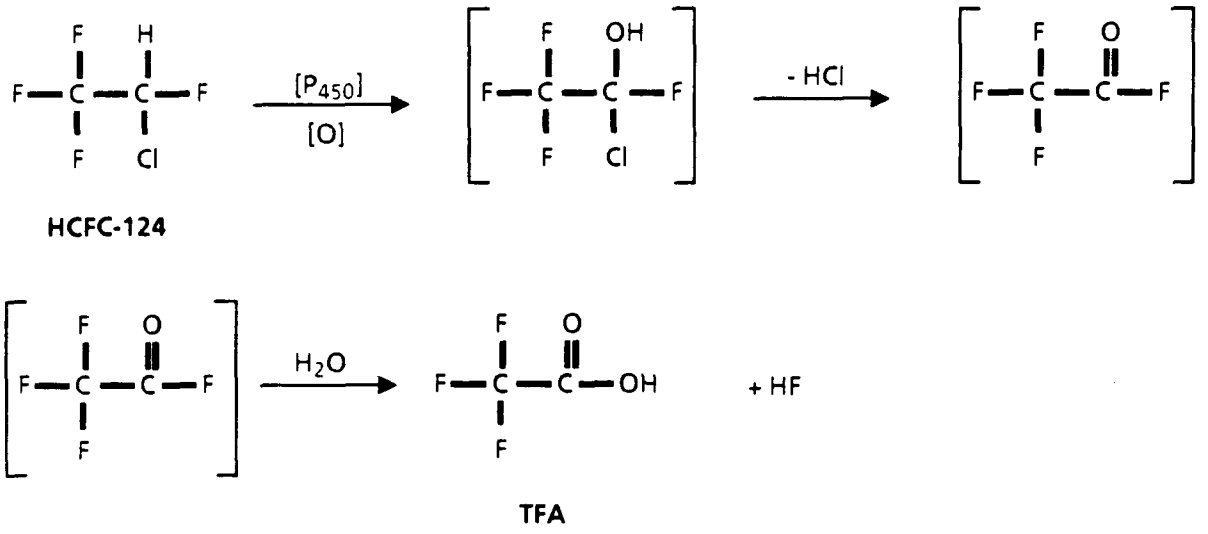


Figure 11. Proposed Metabolic Pathway of HCFC-124. Oxidative metabolism by cytochrome P_{450} yields an unstable geminal halohydrin which releases HCl to form a reactive acylfluoride. The acylfluoride is shown hydrolyzed to TFA and excess fluoride.

Investigation of the metabolism of HCFC-142b did not reveal any reductive metabolites. However, an oxidative metabolite CDFA was found in the urine of both F-344 and Sprague-Dawley rats. The proposed metabolic pathway for HCFC-142b is shown in Figure 12. Here, HCFC-142b is oxidized to 2-chloro-2,2-difluoroethanol by cytochrome P_{450}. The alcohol may then be further oxidized by alcohol and aldehyde dehydrogenases to CDFA. Studies of HCFC-141b (CCl_2FCH_3) have suggested that CDFA is the anticipated oxidative metabolite of HCFC-142b. Metabolic studies of HCFC-141b (Harris and Anders, 1991) detected dichlorofluoroacetic acid in the urine of F-344 rats exposed to 4% HCFC-141b for 4 h. However, at lower exposure levels (1.1% for 2 h), the glucuronide conjugate of 2,2-dichloro-2-fluoroethanol was the only metabolite. This suggests that HCFC-142b may also form a glucuronide conjugate of 2-chloro-2,2-difluoroethanol. The possible formation of a glucuronide conjugate of HCFC-142b was not thoroughly investigated in this study.

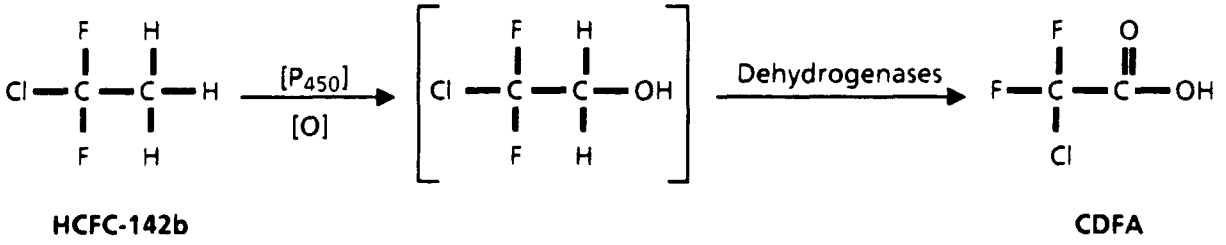
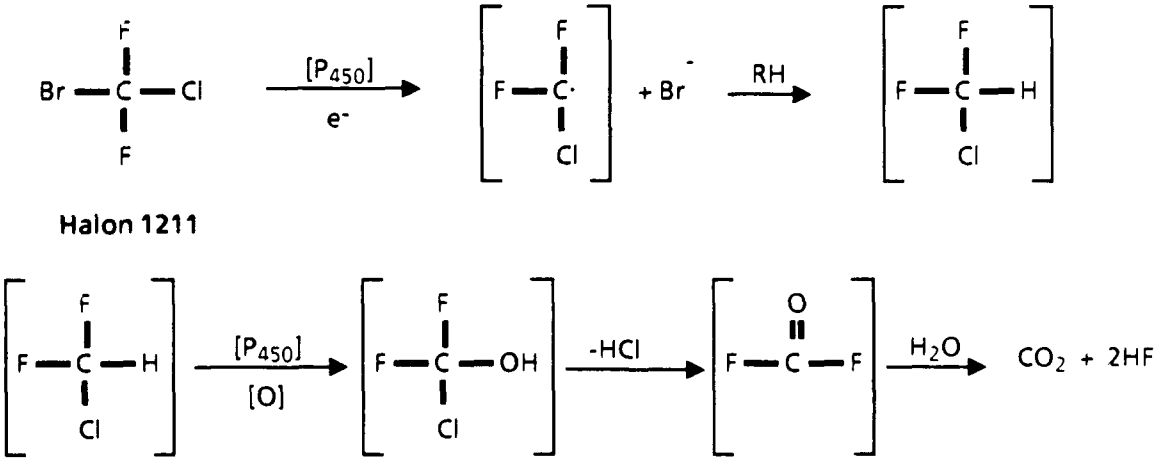


Figure 12. Proposed Metabolic Pathway for HCFC-142b. HCFC-142b is oxidized to 2-chloro-2, 2-difluorethanol by cytochrome P_{450}, which is further metabolized to chlorodifluoroacetic acid.

The exposure studies of PFH did not reveal the presence of any metabolites. Immediately after exposure, PFH was detected in tissues from F-344 and Sprague-Dawley rats. However, 24 h after exposure, PFH was found only in fat. This is consistent with the lack of evidence regarding the metabolism of perfluorinated hydrocarbons. Reports on perfluorochemicals such as perfluorodecalin and perfluorotripropylamine, which are primary components of blood substitutes, have indicated that these compounds are not metabolized (Garrelts, 1990). These substances are primarily expired as a vapor by the lungs. Some of the perfluorocarbons, which are taken up by the reticuloendothelial system, are expired through the lungs at a later time.

The investigation of the metabolism of Halon 1211 did not yield the presence of any metabolites. However, analysis of the urine from F-344 and Sprague-Dawley rats indicated that exposed Sprague-Dawley rats excreted more bromide than the controls. This suggests that Halon 1211 has undergone metabolism, which most probably begins with the loss of bromine. As shown in Figure 13, the proposed metabolism of Halon 1211 begins with reductive dehalogenation. This is followed by oxidative metabolism by cytochrome P_{450}, resulting in the formation of formylfluoride. Formylfluoride would be rapidly hydrolyzed to produce hydrogen fluoride and carbon dioxide. Therefore, increased levels of urinary fluoride would be indicative of oxidative metabolism, and increased levels of urinary bromide would be indicative of metabolic activation through reductive dehalogenation. The toxicological significance of this apparent metabolism of Halon 1211 is unclear. However, these data indicate that Halon 1211 undergoes metabolic activation. This is supported by gas-uptake studies that have shown that Halon 1211 has a V_{max} similar to HCFC-124 (Hoover et al., 1992). Both of these compounds are weakly metabolized and do not appear to produce toxic metabolites that could be detected by the conditions used in this study.



Halon 1211

Figure 13. Proposed Metabolic Pathway for Halon 1211. Reductive metabolism by cytochrome P_{450} yields bromide and chlorodifluoromethane (Freon 22). The oxidation of Freon 22 may yield formylfluoride which would be rapidly hydrolyzed to HF and CO_2.

SECTION 6

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QUALITY ASSURANCE

The study, Metabolite Identification of Halon Replacement Compounds, was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 799. No claim will be made that this was a GLP study as no attempt was made to adhere to the strict requirements of those standards.

The various phases of this study were inspected by members of the Quality Assurance Unit. Results of the inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION	ITEM INSPECTED
June 11, 1991	Metabolite assay data.
August 13, 1991	Inhalation exposure to HCFC-123.
August 14, 1991	Specimen collection and 24 hour post-exposure sacrifice.
August 29, 1991	Inhalation exposure to HCFC-124 and sacrifice.
August 30, 1991	Specimen collection and fluoride analysis.
September 10, 1991	Inhalation exposure to HCFC 142b.
September 26, 1991	Inhalation exposure to Halon 1211.
October 29, 1991	Inhalation exposure to Perfluorohexane and sacrifice.
December 19, 1991	Phase II inhalation exposure to HCFC-123 and sacrifice.
March 24 to April 22, 1992	Data and final report.

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

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Date 27 May 92