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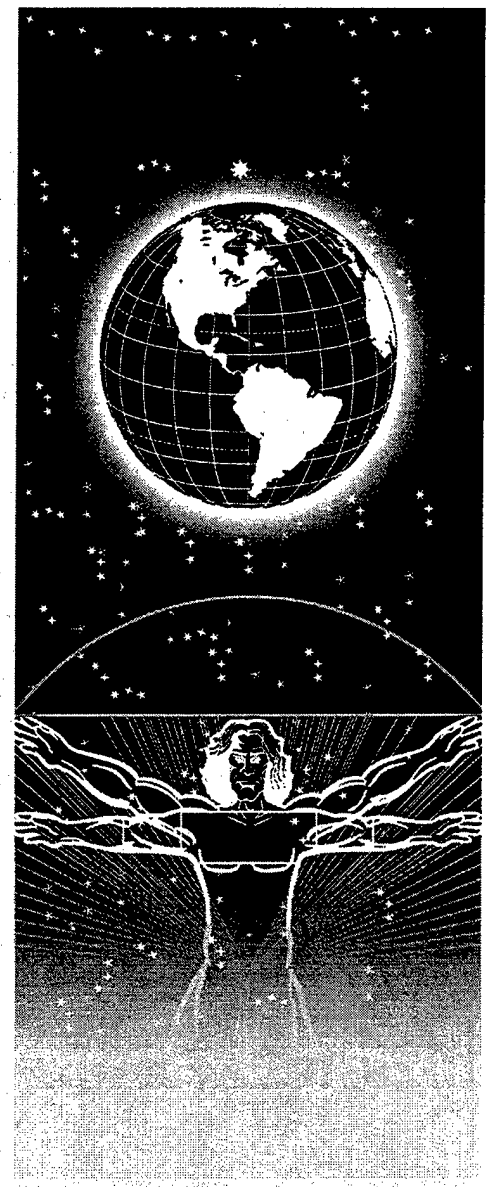

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Gas Uptake Kinetics of 1,1,1,3,3,3-Hexafluoropropane (HFC-236fa) and Identification of Its Potential Metabolites

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NMRI-95-46

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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 Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

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.

FOR THE COMMANDER

Terry A. Childress

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As part of the Navy's efforts to eliminate ozone depleting substances, a new refrigerant, HFC-236fa, has been developed through a joint EPA/Navy effort. This refrigerant is a replacement for refrigerant CFC-114 which is used in centrifugal chillers aboard surface ships and submarines. The aim of these studies was to measure tissue:air partition coefficients and to describe the kinetics of 1,1,1,3,3,3-hexafluoropropane (HFC-236fa) via recirculating gas uptake exposure methods and to look for and identify potential metabolites in blood and urine after inhalation exposure to HFC-236fa. A physiologically based pharmacokinetic (PBPK) model was used to describe mathematically the disposition and metabolism of HFC-236fa employing chemical-specific parameters and apparent whole-body metabolic constants calculated from these experiments. Samples from as uptake studies were collected from rats for the determination of potential metabolites of HFC-236fa in blood, urine and feces. Chamber air samples and moisture trap samples for differing exposure levels and durations were collected and analyzed. Possible metabolites that were considered were hexafluoroacetone, hexafluoropropanol and fluoro-carboxylic acids.

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PREFACE

The research reported herein was conducted by the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., and serves as a final report for the determination of the gas uptake kinetics of 1,1,1,3,3,3-hexafluoropropane (HFC-236fa) and its potential metabolites. The research described in this report began in April 1995 and was completed in June 1995. It was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F38) at the request of the Navy Toxicology Detachment located at Wright Patterson Air Force Base (NMRI/TD) under NMRI Work Unit 132. Lt Col Terry A. Childress served as Contract Technical Monitor for the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, Wright-Patterson Air Force Base, OH. The opinions expressed herein are those of the authors. They are not to be construed as representing the official position of the Departments of the Navy or Air Force. This work is supported by Naval Medical Research and Development Command task no. NAVSEA REIM 1322.

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ABBREVIATIONS

AMU	Atomic mass units
BW	Body weight
°C (or C)	Degrees celsius
cm	Centimeter
F-344	Fischer 344 (rats)
FID	Flame ionization detector
g	Gram
GC	Gas chromatograph(y)
GC/MS	Gas Chromatograph / Mass spectrometer
GI	Gastrointestinal
h(hr)	Hour
hs	Headspace
HFC-236fa	1,1,1,3,3,3-Hexafluoropropane
ID	Inner diameter
L	Liter
m	Meter
min	Minute
mL	Milliliter
mm	Millimeter
msec.	Milliseconds
m/z	Mass to charge ratio (in AMU)
NA	Not applicable
PBPK	Physiologically based pharmacokinetic
ppm	Parts per million
psi	Pounds per square inch
rpm	Rotations per minute
SIM	Selected ion monitoring
TIC	Total ion chromatogram
µg	Microgram
µL	Microliter
µm	Micrometer

SECTION 1
INTRODUCTION

As part of the Navy's efforts to eliminate ozone depleting substances, a new refrigerant, HFC-236fa, has been developed through a joint EPA Navy effort. This refrigerant is a replacement for refrigerant CFC-114, which is used in centrifugal chillers aboard surface ships and submarines. Working closely with EPA's Significant New Alternative Policy (SNAP) program, NAVSEA, the EPA Air and Energy Research Laboratory, and the NMRI Toxicology Detachment have developed the toxicity information needed for approval under the Clean Air Act. A two-year rodent bioassay was proposed to be conducted as a part of the toxicology profile for HFC-236fa. Uptake of this compound is low and indications are that the material is weakly metabolized. In order to assess the need for the bioassay, pharmacokinetic and metabolism studies were conducted to determine the extent of uptake and metabolism. The results of these studies are reported herein.

The aim of these studies was to measure tissue:air partition coefficients and to describe the kinetics of 1,1,1,3,3,3-hexafluoropropane (HFC-236fa), via recirculating gas uptake exposure methods, and to look for and identify potential metabolites in blood and urine after inhalation exposure to HFC-236fa.

Inhalation pharmacokinetics were determined experimentally in Fischer 344 (F-344) male rats. A physiologically based pharmacokinetic (PBPK) model was used to describe mathematically the disposition and metabolism of HFC-236fa employing chemical-specific parameters and apparent whole-body metabolic constants calculated from these experiments.

Samples from gas uptake studies were collected from rats for the determination of potential metabolites of HFC-236fa (1,1,1,3,3,3-hexafluoropropane) in blood, urine, and feces. Chamber air samples and moisture trap samples for differing exposure levels and durations were collected and analyzed. Possible metabolites that were considered were hexafluoroacetone, hexafluoropropanol, and fluoro-carboxylic acids. In order to accommodate the range of volatility of these compounds, both headspace-GC/MS and liquid injection-GC/MS were used. Also both the direct extraction of samples and the derivatization/extraction (for carboxylic acid conversion to methyl esters) were used.

SECTION 2

MATERIALS AND METHODS

TEST CHEMICAL

1,1,1,3,3,3-hexafluoropropane HFC-236fa:

CAS #	690-39-1
Mol. Weight	152
Empirical formula	CF3-CH2-CF3
Boiling point (°C)	-0.7

The chemical was provided by DuPont.

ANIMALS

Male F-344 (200 to 350 g) rats (*Rattus norvegicus*) were obtained from Charles River Breeding Laboratories (Kingston, NY). Animals received Purina Formulab #5008 and softened water *ad libitum*. They were housed in plastic cages (2 to 3/cage) with hardwood chip bedding prior to exposure, and were maintained on a 12-h light/12-h dark light cycle at constant temperature (22 ± 1 °C) and humidity (40 to 60%). Cages were changed twice per week. Animals were marked for identification with a tail tattoo.

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 Human and Health Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

DETERMINATION OF PARTITION COEFFICIENTS

Partition coefficients were determined by using a modified version of the vial-equilibration technique described by Gargas et al. (1989). Whole tissue was harvested and minced into a tissue slurry versus prepared as a tissue homogenate in saline. Rats used to determine partition coefficients were euthanatized with CO_2. Blood was collected from the posterior *vena cava* using a heparinized syringe. Liver (L), muscle (M, quadriceps), fat (F, epididymal and perirenal), and gastrointestinal tract (G, stomach and small intestine) also were removed for analysis. Blood samples (2.0 mL) were placed in 12.4 mL glass vials and incubated/mixed for 3 h at 37 °C with 800 ppm of chemical in the vial headspace. Incubation time was determined by initially exposing samples for 1, 3, 5, or 7 h and observing that no change was seen after 3 h. Chemical concentration was determined by initially using 80, 400, or 800 ppm and observing no difference between 400 and 800 ppm. Whole tissue samples (1.0 g of L and M; 0.50 g of F and G) were minced and incubated/mixed under the same conditions as for blood.

The chemical concentrations in the headspace were analyzed using a HP19395A headspace sampler (Hewlett-Packard, Avondale, PA) connected to a HP5890 gas chromatograph (GC) (Hewlett-Packard, Palo Alto, CA) equipped with a hydrogen flame ionization detector (FID). A 30 m x 0.53 mm Supelco Vo Col™ 3.00 μm Film column was used. Gas chromatography conditions were set with the detector temperature at 250 °C, injection temperature 125 °C, helium carrier gas at 16.0 mL/min column flow plus 24.0 mL/min make-up flow, and oven temperature held constant at 150 °C.

GAS UPTAKE AND METABOLIC CONSTANTS

A closed chamber recirculating gas uptake system with a volume of 8.0 L was used for the estimation of whole animal metabolic constants (V_{maxc}, K_m, and K_{fc}) (Gargas et al., 1986). Initially loss runs without rats were completed to determine the loss rate of the chemical from the system alone. Then loss runs were performed with dead rats to determine if there was any additional loss by adherence to the surface of the animals. Next the actual uptake runs were performed in which three F-344 rats were exposed to the study chemical. Five exposure concentrations were performed for 6 h each (HFC-236fa concentrations were 100, 530, 2350, 7300, and 18000 ppm). Barilyme (75 g) was used as the CO_2 absorber. Oxygen concentrations were maintained at (21% ± 1) during the exposures. The system flow was maintained at 2.5 L/min with the flow to the sample loop of the GC at 100 mL/min.

The chemical concentrations in the chamber atmosphere were monitored every 5 min for the first 30 min and every 15 min thereafter using a gas sampling valve connected to a HP5890 GC. Chromatography was performed on a 6' x 1/8" 3% OV-17, 60/80 Chromosorb column. The GC was equipped with a hydrogen FID with temperature set at 250 °C, helium carrier flow at 35.4 mL/min, injection temperature of 100 °C, and oven temperature held constant at 105 °C.

MODEL DEVELOPMENT

SIMUSOLV (DOW Chemical Co., Midland, MI), a Fortran-based continuous simulation language with optimization capabilities, was used on a VAX/VMS 8530 mainframe computer (Digital Equipment Corp., Maynard, MA). The general form of the PBPK model (Figure 1) followed that of Ramsey and Andersen (1984). The codes that made up the PBPK models are given in the Appendices. Parameters were optimized by SIMUSOLV which used the log likelihood function as the criterion, and either the generalized reduced gradient method for single parameter optimization or the Nelder-Mead search method for multiple parameters optimization to adjust the values.

Physiological constants for calculating volumes of the compartments are shown in Table 1. Tissue volume constants are scaled to the actual body weight (BW) of the rats under study (fat volume was derived from Anderson et al. [1993]); other constants were according to Linstedt (Physiological Parameters Working Group, ILSI Risk Science Institute, unpublished data). Blood flows are expressed as a percentage of cardiac output which was scaled to BW to the exponent 0.75. Alveolar ventilation is also scaled to BW to the exponent 0.75. Cardiac output and alveolar ventilation, based on those described by Gargas et al. (1986) for resting animals, are summarized in Table 1.

Blood:air and tissue:air partition coefficients were obtained as described above. Metabolic constants were determined using the model to obtain a simultaneous fit to the closed chamber gas uptake data. The constants are scaled to BW using the allometric relationship described by Andersen et al. (1987).

TABLE 1. KINETIC CONSTANTS AND PHYSIOLOGICAL PARAMETERS USED IN PBPK MODELING IN RATS

Description	[Units] Parameter
Tissue Volumes	[Fraction of Body Weight:BW]
Liver	$V_LC = 0.037$
Fat	$V_FC = 0.01*(35*BW+2.1)$
GI Tract	$V_GC = 0.033$
Slowly Perfused	$V_SC = 0.558$
Rapidly Perfused	$V_RC = 0.031$
Flow Rates	[L/h/kg]
Alveolar Ventilation	$Q_PC = 14.0$
Cardiac Output	$Q_CC = 14.0$
	[Fraction of Cardiac Output]
Liver	$Q_LC = 0.032$
Fat	$Q_FC = 0.058$
GI Tract	$Q_GC = 0.183$
Slowly Perfused	$Q_SC = 0.255$
Rapidly Perfused	$Q_RC = 0.472$

PBPK MODEL CONSTRUCTION

Figure 1 shows the scheme of the PBPK model, essentially as described by Ramsey and Andersen (1984). An additional compartment was added to describe the gastrointestinal (GI) tract.

Mass transfer differential equations describing each compartment of the PBPK model for both chemicals (schematically shown in Figure 1) are presented below.

For simple, well-stirred compartments in which neither metabolism or other losses occurred (rapidly and slowly perfused tissues, fat, and gut), the change in the amount of chemical (A_i) over time (t) was described as follows:

$$dA_i/dt = Q_i(CA - CV_i)$$

where subscript $_i$ represents "i-th" compartment; Q_i represents the blood flow through the "i-th" compartment; CA represents the arterial concentration; CV_i represents the venous concentration leaving the "i-th" compartment ($CV_i = C_i/P_i$; where C_i is a concentration in the tissue in "i-th" compartment and P_i is the tissue/blood partition coefficient for "i-th" compartment. $C_i = A_i/V_i$, where V_i represents the volume of the "i-th" compartment).

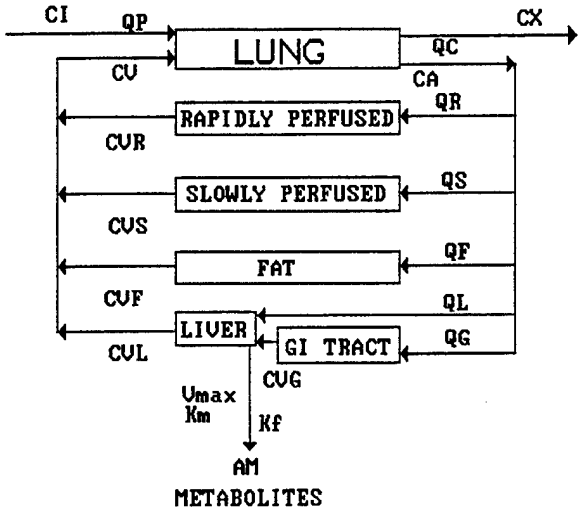


Figure 1. A scheme of PBPK model used for computer simulations of HFC-236fa disposition and metabolism in rats.

For the liver compartment, a loss term (RAM) was added to the well-stirred compartment description to account for rate of metabolism ($RAM = V_{max}CV_L/(K_m + CV_L) + K_fCV_LV_L$; where V_{max} is apparent-maximal velocity rate of the metabolism, CV_L is venous concentration leaving the liver, K_m is apparent Michaelis-Menten constant, K_f is the first order rate of metabolism, and V_L is the volume of liver):

$$dA_L/dt = Q_L(CA - CV_L) + Q_G(CV_G - CV_L) - RAM$$

where Q_G is the blood flow through the portal circulation (from the GI tract) and CV_G is a concentration of the chemical that reaches the liver via portal circulation (from the GI tract). Units for the above variables are as follows: amounts - mg, concentrations - mg/L, flows - L/h, and rates - mg/h. The actual codes used for computer simulation of HFC-236fa are included in APPENDIX A.

METABOLITE IDENTIFICATION

EXTRACTION AND ESTERIFICATION/EXTRACTION GC/MS ANALYSES

Simple extractions of either 0.5 mL or 0.5 g of samples with 1.0 mL hexane or cyclohexane and vortexed for 1 h at 40 °C were made. Both solvents were used in order to permit examination of the chromatographic regions obscured by either solvent. The organic layer was then transferred to autosampler vials for analysis.

Esterification of potential carboxylic acids present in the samples was achieved by taking 0.1 mL or 0.1 g of sample, adding 0.5 mL sulfuric acid and 0.1 mL dimethyl sulfate, and vortexing the samples for 30 min at 60 °C. Extraction of these samples was achieved by adding either 1.0 mL hexane or cyclohexane, vortexing the samples for 60 min at 37 °C, and then centrifuging the samples for 12 min at 2500 rpm. The organic layer was then transferred to autosampler vials for analysis.

Samples were analyzed using either the full scan or the selected ion monitoring modes of operation with the following analytical instruments:

Hewlett-Packard 5971A Series Mass Selective Detector

Hewlett-Packard 5890A Series II Gas Chromatograph

Column: DB-624, 30 m X 0.25 mm ID, 1.4 μm film thickness

Program: 35 °C - 11.7 min/ 15 °C per min/ 180 °C - 5.0 min

Scan Range: 35-200 amu

SIM: 51, 69, 99, 147 and 59, 63, 69, 100 ions

Summaries of the operation parameters for this analysis are given in Figures 2, 3, and 4.

METHOD INFORMATION FOR: C:\HPCHEM\1\METHODS\WTBSCAN.M

COMMENTS: 0.8PSI WITH CONSTANT FLOW AT 35 C

GENERAL INFORMATION

INLET:	GC
TUNE FILE:	ATUNE.U
ACQUISITION MODE:	SCAN

MS INFORMATION

SOLVENT DELAY:	0.0 MIN
EMV OFFSET:	294.1
RESULTING VOLTAGE:	1835.3

(Scan Parameters)

LOW MASS:	35 AMU
HIGH MASS:	200 AMU
THRESHOLD:	500
SAMPLING # :	2
A/D SAMPLES:	4

(Real Time Plot Parameters)

TIME WINDOW:	12 MIN
PLOT 1 TYPE:	TOTAL ION
PLOT 2 TYPE:	NO PLOT

(Inlet B Temperature Program Information)

OVEN TRACK:	OFF
INITIAL TEMPERATURE:	80 C
INITIAL TIME:	60 MIN

(Inlet B Flow Settings)

COLUMN LENGTH:	30 m
COLUMN DIAMETER:	0.25 mm
GAS:	HELIUM
VACUUM COMPENSATION:	ON
PRESSURE:	0.8 PSI
FLOW:	1.5 mL/min
LINEAR VELOCITY:	49.3 cm/sec.
SPLIT FLOW:	1 mL/min
SPLIT RATIO:	0.6

GC INFORMATION

(GC Zone Information)

INJECTION PORT B:	80 C
DETECTOR B:	200 C
AUXILLARY:	OFF

(Oven Parameters)

EQUILIBRIUM TIME:	
OVEN MAXIMUM:	240 C
OVEN:	ON
CRYOGENICS:	OFF
AMBIENT:	25 C
INITIAL TEMPERATURE:	35 C
INITIAL TIME:	11.66 MIN
RATE (C/ MIN):	15
FINAL TEMPERATURE:	180 C
FINAL TIME:	5.0 MIN
RUN TIME:	26.33 MIN

(Injector Information)

INJECTION SOURCE:	AUTOMATIC
INJECTION LOCATION:	REAR
SAMPLE WASHES:	4
SAMPLE PUMPS:	3
SAMPLES VOLUME:	2 STOPS
VISCOSITY DELAY:	0
SOLVENT B WASHES:	3
ON COLUMN:	NO
PURGE B:	ON

TIMED ENTRIES

DETECTOR OFF:	7.24 MIN
DETECTOR ON:	9.26 MIN

FIGURE 2. GC/MS Acquisition Information for Hexane Extractions. This is a summary of full scan operation parameters for the Hewlett-Packard 5971A GC/MS as used for the analysis of the samples undergoing extraction and/or esterification and extraction using hexane.

METHOD INFORMATION FOR: C:\HPCHEM\1\METHODS\WTBCYCL.M

COMMENTS: 0.8PSI WITH CONSTANT FLOW AT 35 C

GENERAL INFORMATION

INLET:	GC
TUNE FILE:	ATUNE.U
ACQUISITION MODE:	SCAN

MS INFORMATION

SOLVENT DELAY:	0.0 MIN
EMV OFFSET:	294.1
RESULTING VOLTAGE:	1835.3

(Scan Parameters)

LOW MASS:	35 AMU
HIGH MASS:	200 AMU
THRESHOLD:	500
SAMPLING # :	2
A/D SAMPLES:	4

(Real Time Plot Parameters)

TIME WINDOW:	12 MIN
PLOT 1 TYPE:	TOTAL ION
PLOT 2 TYPE:	NO PLOT

(Inlet B Temperature Program Information)

OVEN TRACK:	OFF
INITIAL TEMPERATURE:	80 C
INITIAL TIME:	60 MIN

(Inlet B Flow Settings)

COLUMN LENGTH:	30 m
COLUMN DIAMETER:	0.25 mm
GAS:	HELIUM
VACUUM COMPENSATION:	ON
PRESSURE:	0.8 PSI
FLOW:	1.5 mL/min
LINEAR VELOCITY:	49.3 cm/sec.
SPLIT FLOW:	1 mL/min
SPLIT RATIO:	0.6

GC INFORMATION

(GC Zone Information)

INJECTION PORT B:	80 C
DETECTOR B:	200 C
AUXILLARY:	OFF

(Oven Parameters)

EQUILIBRIUM TIME:	
OVEN MAXIMUM:	240 C
OVEN:	ON
CRYOGENICS:	OFF
AMBIENT:	25 C
INITIAL TEMPERATURE:	35 C
INITIAL TIME:	11.66 MIN
RATE (C/ MIN):	15
FINAL TEMPERATURE:	180 C
FINAL TIME:	5.0 MIN
RUN TIME:	26.33 MIN

(Injector Information)

INJECTION SOURCE:	AUTOMATIC
INJECTION LOCATION:	REAR
SAMPLE WASHES:	4
SAMPLE PUMPS:	3
SAMPLES VOLUME:	2 STOPS
VISCOSITY DELAY:	0
SOLVENT B WASHES:	3
ON COLUMN:	NO
PURGE B:	ON

TIMED ENTRIES

DETECTOR OFF:	12.80 MIN
DETECTOR ON:	14.10 MIN

FIGURE 3. GC/MS Acquisition Information for Cyclohexane Extractions. This a summary of full scan operation parameters for the Hewlett-Packard 5971A GC/MS as used for the analysis of the samples undergoing extraction and/or esterification and extraction using cyclohexane.

METHOD INFORMATION FOR: C:\HPCHEM\1\METHODS\WTBSIM1.M

COMMENTS: 0.8PSI WITH CONSTANT FLOW AT 35 C

GENERAL INFORMATION

INLET:	GC
TUNE FILE:	ATUNE.U
ACQUISITION MODE:	SIM

MS INFORMATION

SOLVENT DELAY:	0.20 MIN
EMV OFFSET:	294.1
RESULTING VOLTAGE:	1835.3

(SIM Parameters)

GROUP 1 IONS:	59, 63, 69, 100
	or 59, 69, 99, 147
DWELL PER ION:	200 msec.
LOW RESOLUTION:	NO
GROUP START TIME:	1.00 MIN

(Real Time Plot Parameters)

TIME WINDOW:	13 MIN
PLOT 1 TYPE:	59 ION
PLOT 2 TYPE:	69 ION

(Inlet B Temperature Program Information)

OVEN TRACK:	OFF
INITIAL TEMPERATURE:	80 C
INITIAL TIME:	60 MIN

(Inlet B Flow Settings)

COLUMN LENGTH:	30 m
COLUMN DIAMETER:	0.25 mm
GAS:	HELIUM
VACUUM COMPENSATION:	ON
PRESSURE:	0.8 PSI
FLOW:	1.5 mL/min
LINEAR VELOCITY:	49.3 cm/sec.
SPLIT FLOW:	1 mL/min
SPLIT RATIO:	0.6

GC INFORMATION

(GC Zone Information)

INJECTION PORT B:	80 C
DETECTOR B:	200 C
AUXILLARY:	OFF

(Oven Parameters)

EQUILIBRIUM TIME:	
OVEN MAXIMUM:	240 C
OVEN:	ON
CRYOGENICS:	OFF
AMBIENT:	25 C
INITIAL TEMPERATURE:	35 C
INITIAL TIME:	11.66 MIN
RATE (C/ MIN):	15
FINAL TEMPERATURE:	180 C
FINAL TIME:	5.0 MIN
RUN TIME:	26.33 MIN

(Injector Information)

INJECTION SOURCE:	AUTOMATIC
INJECTION LOCATION:	REAR
SAMPLE WASHES:	4
SAMPLE PUMPS:	3
SAMPLES VOLUME:	2 STOPS
VISCOSITY DELAY:	0
SOLVENT B WASHES:	3
ON COLUMN:	NO
PURGE B:	ON

TIMED ENTRIES

(for Hexane solvent)

DETECTOR OFF:	7.24 MIN
DETECTOR ON:	9.26 MIN

(for Cyclohexane solvent)

DETECTOR OFF:	12.80 MIN
DETECTOR ON:	14.10 MIN

FIGURE 4. GC/MS Acquisition Information for SIM Operation. This is a summary of selected ion operation parameters for the Hewlett-Packard 5971A GC/MS as used for the analysis of the samples undergoing extraction and/or esterification and extraction using either Hexane or Cyclohexane.

HEADSPACE GC/MS ANALYSES

After some experimentation to determine optimum conditions for the headspace autosampler, 0.5 mL (0.5 g) or less aliquots of samples were transferred to 9 mL headspace vials and analyzed using the full scan mode of operation with the following analytical instruments:

Hewlett-Packard 5970B Series Mass Selective Detector
Hewlett-Packard 5890A Gas Chromatograph
Tekmar 7000 Headspace Analyzer & Cryofocusing Module
Column: Poraplot Q, 30 m X 0.32 mm ID, 10 µm film thickness
Program: 45 °C - 10.0 min/ 12 °C per min/ 175 °C - 5.0 min
Scan Range: 47-500 amu

Summaries of the operation parameters for this analysis are given in figures 5 and 6.

REAGENTS AND STANDARDS

The dimethyl sulfate, methyl trifluoroacetate, methyl pentafluoropropionate, pentafluoropropionic acid, 1,1,1,3,3,3-hexafluoro-2-propanol, 1,3-difluoro-2-propanol, 2,2,3,3,3-pentafluoro-1-propanol, and hexafluoroacetone•trihydrate were obtained from Aldrich Chemical Co. (Milwaukee, WI). The sulfuric acid, cyclohexane, and hexane were obtained from Fisher Scientific (Fair Lawn, NJ).

EQUIPMENT

All samples were prepared and analyzed using the following instrumentation:
Hewlett-Packard 5971A Series Mass Selective Detector, Serial No. 3324A04160
Hewlett-Packard 5890A Series II Gas Chromatograph, Serial No. 3310A49086
Hewlett-Packard Vectra 486/66U Data System, Serial No. 3328A52882
Hewlett-Packard 7673 Autosampler/GC Injector, Serial No. 3329A35598
Hewlett-Packard 18596B Sample Tray, Serial No. 3329A32579
Tekmar Cryofocusing Module, Serial No. 92176014
Tekmar 7000 Headspace Analyzer, Serial No. 92063014
Hewlett-Packard 5970B Series Mass Selective Detector, Serial No. 2623a01335
Hewlett-Packard 5890A Gas Chromatograph, Serial No. 2623A07142
Hewlett-Packard Vectra 386/25 Data System, Serial No. 312A091695
Haake-Buchler HBI Vortex-Evaporator, Model 4322000
IEC Centra-8R Centrifuge, Serial No. 24780720

METHOD INFORMATION FOR: C:\CHEMPC\METHODS\HEADSCAN.M

GENERAL INFORMATION

INLET:	GC
TUNE FILE:	ATUNE.U
ACQUISITION MODE:	SCAN

MS INFORMATION

SOLVENT DELAY:	2.70 MIN
EMV OFFSET:	-200
RESULTING VOLTAGE:	1800

(Scan Parameters)

LOW MASS:	47 AMU
HIGH MASS:	500 AMU
THRESHOLD:	500
SAMPLING # :	2
A/D SAMPLES:	4

(Real Time Plot Parameters)

TIME WINDOW:	30 MIN
PLOT 1 TYPE:	TOTAL ION
PLOT 2 TYPE:	NA

(Inlet A Temperature Program Information)

OVEN TRACK:	OFF
INITIAL TEMPERATURE:	200°C
INITIAL TIME:	NA

(Inlet A Flow Settings)

COLUMN LENGTH:	30 m
COLUMN DIAMETER:	0.53 mm
GAS:	HELIUM
VACUUM COMPENSATION:	ON
FLOW :	10.3 mL/min

GC INFORMATION

(GC Zone Information)

INJECTION PORT B:	80 C
DETECTOR B:	200 C
AUXILLARY:	OFF

(Oven Parameters)

EQUILIBRIUM TIME:	0.25 MIN
OVEN MAXIMUM:	250 C
OVEN:	ON
CRYOGENICS:	NA
AMBIENT:	25 C
INITIAL TEMPERATURE:	45 C
INITIAL TIME:	10.0 MIN
RATE (C/ MIN):	12
FINAL TEMPERATURE:	175 C
FINAL TIME:	5.0 MIN
RUN TIME:	25.83 MIN

(Injector Information)

INJECTION SOURCE:	MANUAL
INJECTION LOCATION:	NA
SAMPLE WASHES:	NA
SAMPLE PUMPS:	NA
SAMPLES VOLUME:	NA
VISCOSITY DELAY:	NA
SOLVENT B WASHES:	NA
ON COLUMN:	NA
PURGE A:	ON
PURGE TIME:	1.70 MIN

FIGURE 5. GC/MS Acquisition Information for Headspace Operation. This is a summary of full scan operations parameters for the Hewlett-Packard 5970B GC/MS as used for the analysis of the headspace samples.

TEKMAR 7000 HEADSPACE AUTOSAMPLER PARAMETERS

Platen	65°C			Platen Equilibration:	0.10min
Sample Vial:	10 mL			Sample	5.0 min
Mixer: ON		Mix:	min	Mix Power:	
Stabilize:	1.0 min			Cap Cooldown:	-10°C
Pressure:	1			Press Equilibration:	0.10 min
Loop:	1.5 min			Loop Equilibration:	0.25 min
Inject:	1.5 min			Cap Injection:	1.0/100°C
Valve:	100°C			Line:	100°C
Capillary Union	NO			Injection per Vial:	1
GC cycle time:	25 min			Parameter	OFF

FIGURE 6. Tekmar 7000 Headspace Autosampler Parameters. This is a summary of the operation parameters for the Tekmar 7000 as used for the loading and injecting of headspace samples.

SECTION 3

RESULTS

PARTITION COEFFICIENTS

The rat tissue:air partition coefficients determined for HFC-236fa, which were used in the PBPK model optimization, are shown in Table 2.

TABLE 2. PARTITION COEFFICIENTS FOR HFC-236fa IN RATS

Partition Coefficients		Ratio ± S.D. (n= 12)
Blood:air	PB	0.49 ± 0.04
Liver:air	PLA	0.56 ± 0.06
Fat:air	PFA	3.69 ± 0.56
Gut:air	PGA	0.56 ± 0.06
Rapidly perfused/air	PRA	0.56 ± 0.06
Slowly perfused/air	PSA	0.87 ± 0.08

LOSS RUNS

Results of loss runs with and without animals are shown in Table 3. When the percent loss per hour for a 20,000 ppm HFC-236fa six-hour exposure of three live rats was calculated, a loss rate of 1.59% per hour resulted. This was nearly four times the average percent loss per hour for loss runs at this concentration (0.40 ± 0.06% per hour), either with or without dead rats. This increase in loss rate was also true for 10,000 ppm HFC-236fa. Lower concentrations however, such as 100 ppm and 500 ppm, yielded a larger increase in loss rate.

TABLE 3. SUMMARY OF LOSS RATES[†]

Nominal Chamber Concentration of HFC-236fa	Percent loss per hour during loss runs	Percent loss per hour during exposures
100 ppm	2.85 ± 1.40% per hour	11.77% per hour
500 ppm	0.67% per hour	8.93% per hour
5,000 ppm	n/a	1.57% per hour
10,000 ppm	0.38 ± 0.07% per hour	2.00% per hour
20,000 ppm	0.40 ± 0.06% per hour	1.59% per hour

[†]Percent loss per hour calculated for loss runs includes loss runs with and without dead rats as there was no significant difference in rates. For 100 ppm and 20,000 ppm, n=3. For 10,000 ppm, n=4. For 500 ppm, n=1.

GAS UPTAKE STUDIES

The inhalational uptake of HFC-236fa by the rat showed two discernible phases: a rapid equilibration phase that lasted up to 30 minutes followed by a slow linear uptake phase (Figure 2). The continuous lines represent the simulations with the assumption of no metabolism and with appropriate chamber loss rates included. The individual points represent the actual gas uptake data collected with live animals in the chamber.

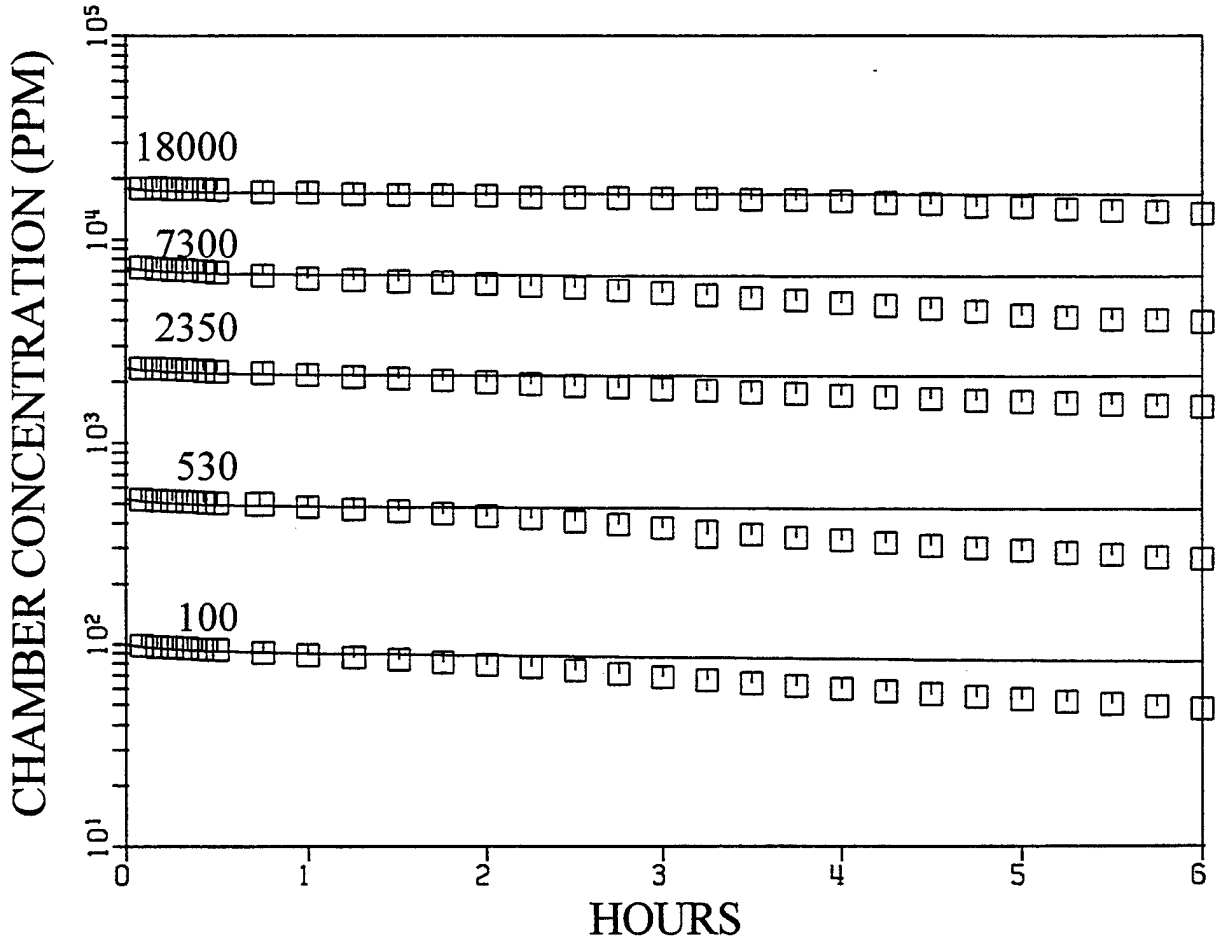


FIGURE 7. HFC-236FA GAS UPTAKE

METABOLITE IDENTIFICATION

Samples were designated as being from either 6 h of exposure or 24 h postexposure. Simple extractions of one 24-h rat blood, one 24-h rat urine, one 24-h rat feces, one rat blood control, and one rat urine control, were extracted with hexane and again with cyclohexane as the solvents. One 6-h rat blood, three 24-h rat bloods, three 24-h rat urines, three 24-h rat feces, one rat blood control, one rat urine control, and one water blank were esterified and extracted with hexane as the solvent. In addition, one 24-h rat blood, one 24-h -rat urine, one 24-h rat feces, one rat blood control, one rat urine control, and one water blank were esterified and extracted with cyclohexane as the solvent.

No potential fluorocarbon metabolites were detected in these samples, and no response to a single target ion, such as the 69 ion, was detected in these samples that could not also be found in a blank or control sample. In addition, standards of related fluorocarbons were obtained and solutions of these were analyzed in order to "map out" the chromatographic ranges and provide typical mass spectra likely for any metabolites. See Table 4.

TABLE 4. *FLUOROCARBON STANDARDS DATA*

STANDARD	retention time DB-624	MAJOR IONS			
methyl trifluoroacetate	3.7 min	50	59	69	99
methyl pentafluoropropionate	4.45 min	59	69	100	119
1,3-difluoro-2-propanol	15.2 min	43	63		
pentafluoropropanol	12.9 min	43	69	89	100
1,1,1,3,3,3-hexafluoro-2-propanol	16.6 min	51	69	79	99
hexafluoroacetone •trihydrate	----	50	69	97	147
pentafluoropropionic acid	----	45	69	100	119
HFC-236fa	2.5 min	64	69	113	133

A chromatogram indicating the retention times of five of these standards is shown in figure 8. Analytical single ion monitoring runs of solutions of the standards yielded detection limits of 0.5μg/mL for methyl trifluoroacetate and 0.2μg/mL for methyl pentafluoropropionate. Curves that were generated for the fluoro-alcohols indicated a quantitation limit of 0.5-5μg/mL. Since the esterified sample represents a ten-fold dilution (0.1mL sample → 1mL solvent), the effective detection limit would be in a range of 5-50μg/mL.

Samples of chamber air, rat blood, rat urine, rat feces, and moisture trap water from various exposure levels were analyzed by headspace GC/MS. The only fluorocarbon compound detected was the HFC-236fa itself at a retention time of 17.2 min, as verified with air samples from the inhalation system. Copies of typical chromatograms, mass spectra, and library matches from

these analyses are given in Figures 9 through 12. Examples of the results from four particular exposures are given in Table 5 , as follows:

TABLE 5. HEADSPACE SAMPLE RESULTS

Exposure date	Exposure concentration	Samples	HFC-236fa Area counts
26-Apr-95	20380 ppm	Feces sample collected postexposure	0
4-May-95	24460 ppm	Blood sample collected postexposure	74,963
4-May-95	24460 ppm	Blood sample collected postexposure	117,561
24-May-95	17820 ppm	Chamber air sample collected postexposure	6,848,743
24-May-95	17820 ppm	Water collected in moisture trap during exposure	1,989,869
24-May-95	17820 ppm	Blood sample collected postexposure	298,344
24-May-95	17820 ppm	Blood sample collected postexposure	246,587
24-May-95	17820 ppm	Urine sample collected postexposure, postmortem via bladder puncture	0
24-May-95	17820 ppm	Urine sample collected postexposure, postmortem via bladder puncture	0
24-May-95	17820 ppm	Water collected in moisture trap during exposure	2,193,195
26-May-95	2335 ppm	Urine sample collected postexposure, postmortem via bladder puncture	112,112
26-May-95	2335 ppm	Urine sample collected postexposure, postmortem via bladder puncture	0
26-May-95	2335 ppm	Headspace sample from above urine sample	36,884

It should be noted that for the results of the 26-May-95 exposure, only urine #1 was analyzed on 26-May-95. In order to check on the feasibility of storing samples, on 29-May-95 the frozen sample of 26-May-95 was reanalyzed and yielded nothing. A 3 mL aliquot of headspace air above the urine was transferred to a 9 mL headspace vial and yielded a peak of HFC-236fa close to 1/3 the previously found area. The samples had been kept frozen at -20 °C after the initial analysis, but there appears to be problems associated with storage and transferring to the analytical vials.

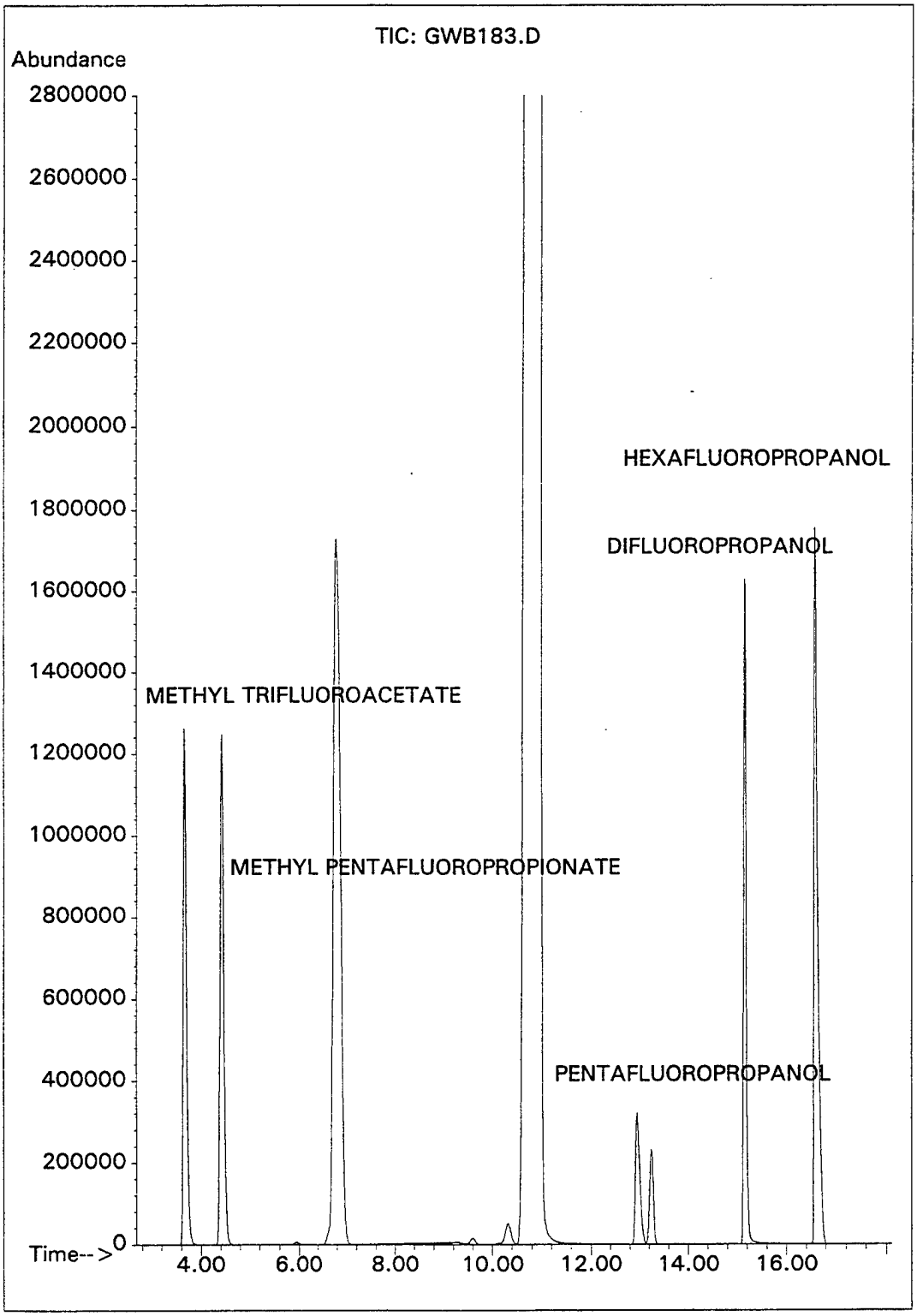


FIGURE 8. Chromatogram of Fluorocarbon Standards. Chromatographic separations of standards were achieved using a DB-624 column which is programmed at 35 C - 11.77 min/15 C per min/ 180 C - 5min. Retention times for the standards are 3.7 min for methyl trifluoroacetate, 4.45 min for methyl pentafluoropropionate, 12.9 min for pentafluoropropanol, 15.2 min for 1,3-difluoro-2-propanol, and 16.6 min for 1,1,1,3,3,3-hexafluoro-2-propanol. The remaining peaks are alkane components of the hexane solvent.

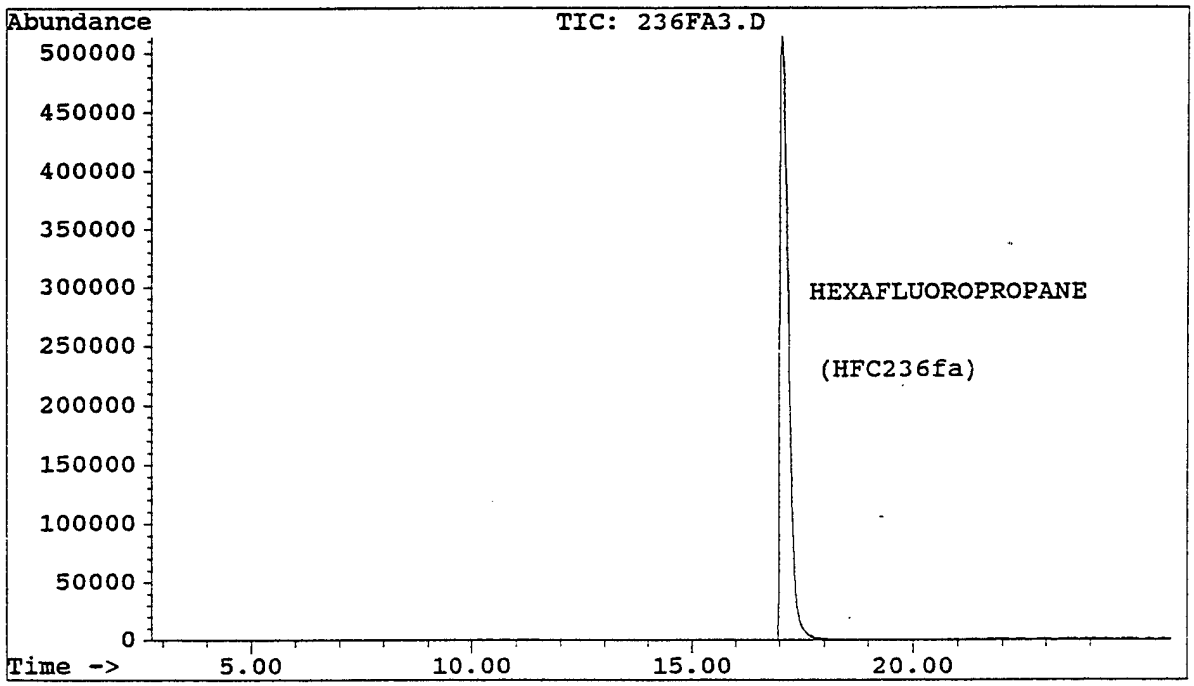
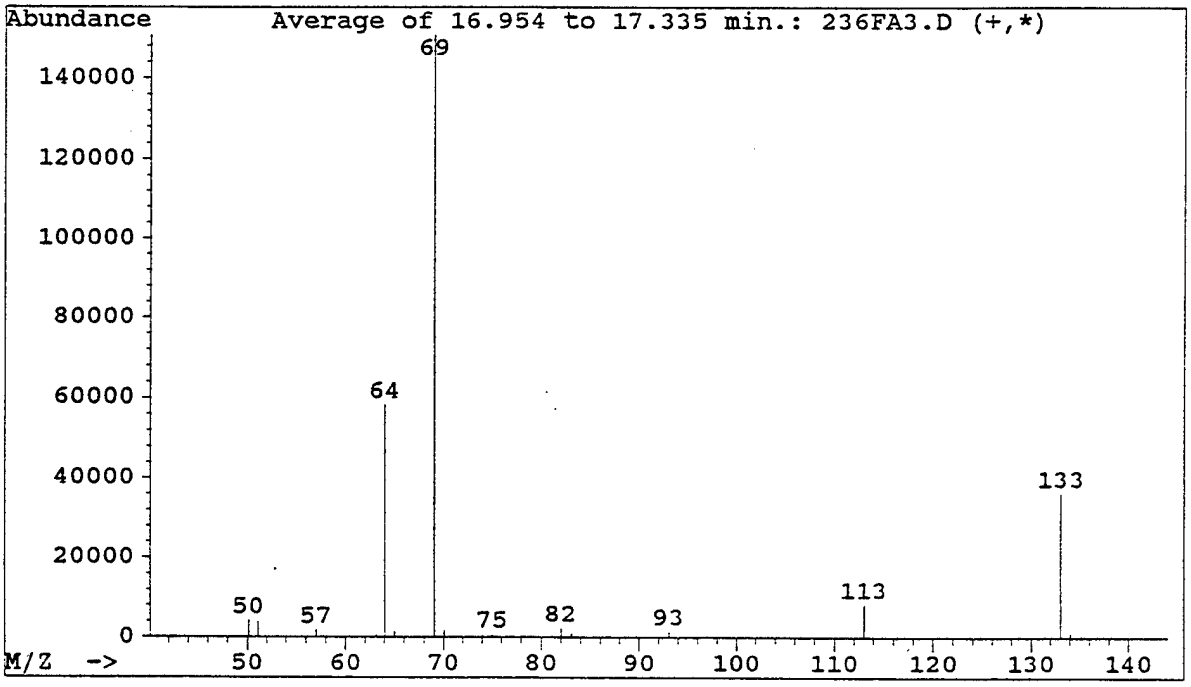


Figure 9. Chromatogram and Mass Spectrum for Headspace Air Chamber Sample. The top graph is a chromatogram of a 1/9 dilution in air of a 13600 ppm HFC-236fa air chamber sample with separation achieved using a Poraplot Q column, which is programmed at 45 °C - 10.0 min/ 12° C per min/ 175° C - 5.0 min. The retention time of HFC-236fa is ~17.2 min The lower graph is a mass spectrum of the peak at 17.2 min with major ions of 64, 69, 113, and 133.



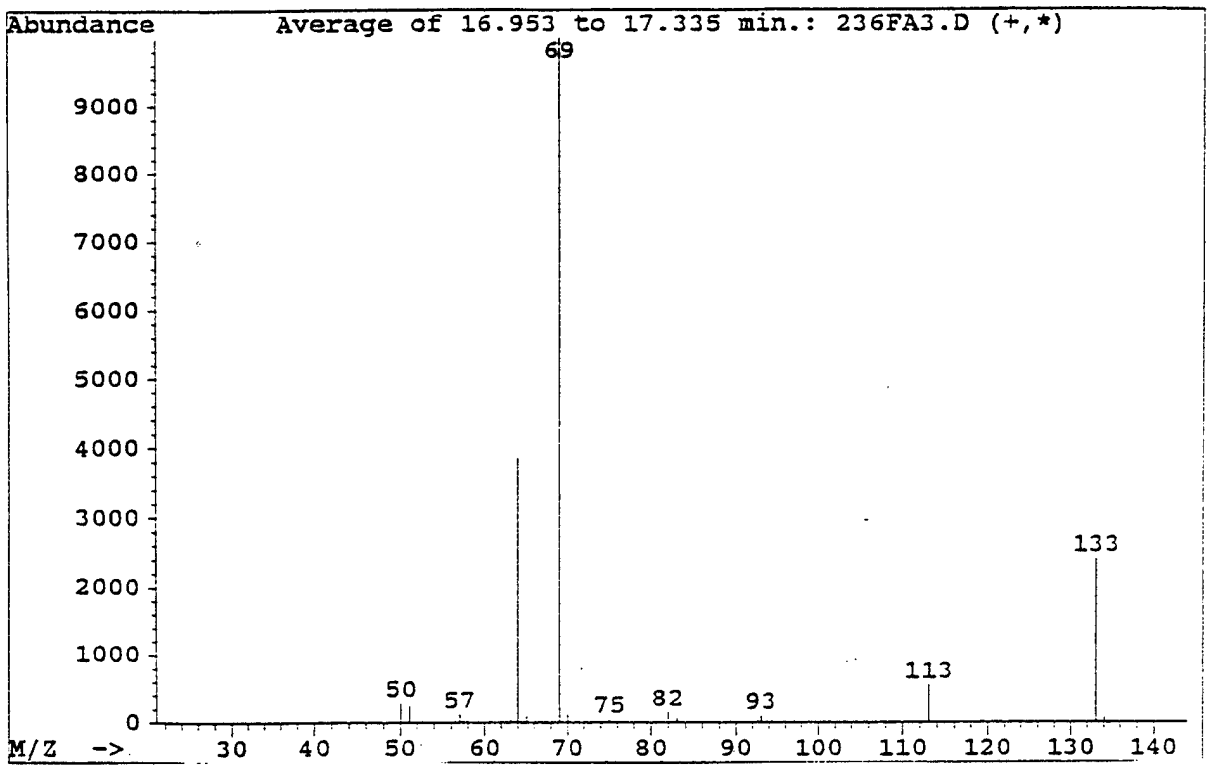
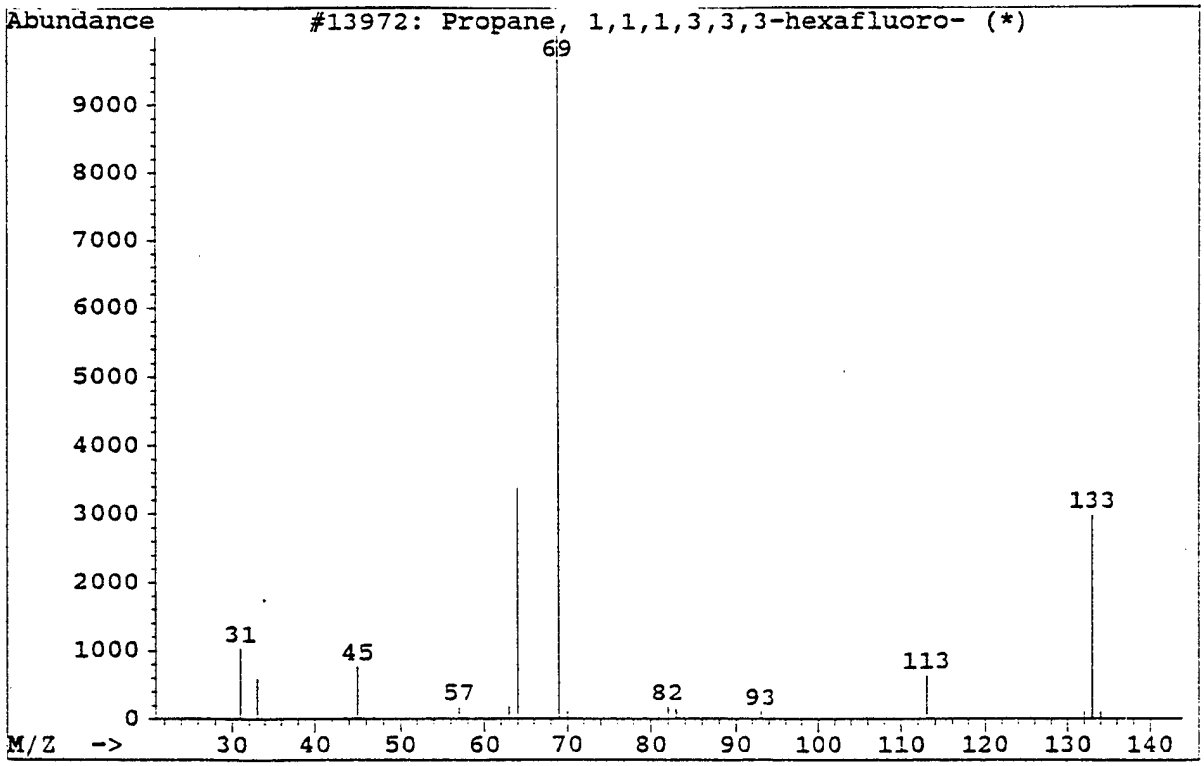


Figure 10. Mass spectra and library match of HFC-236fa. The top graph is a mass spectrum of the chromatographic peak at ~17.2 min from the air chamber sample. The lower graph is the mass spectrum of 1,1,1,3,3,3-hexafluoropropane from the Wiley library of mass spectra, as matched by the Hewlett-Packard GC/MS software, with a quality match of 74 (out of 100).



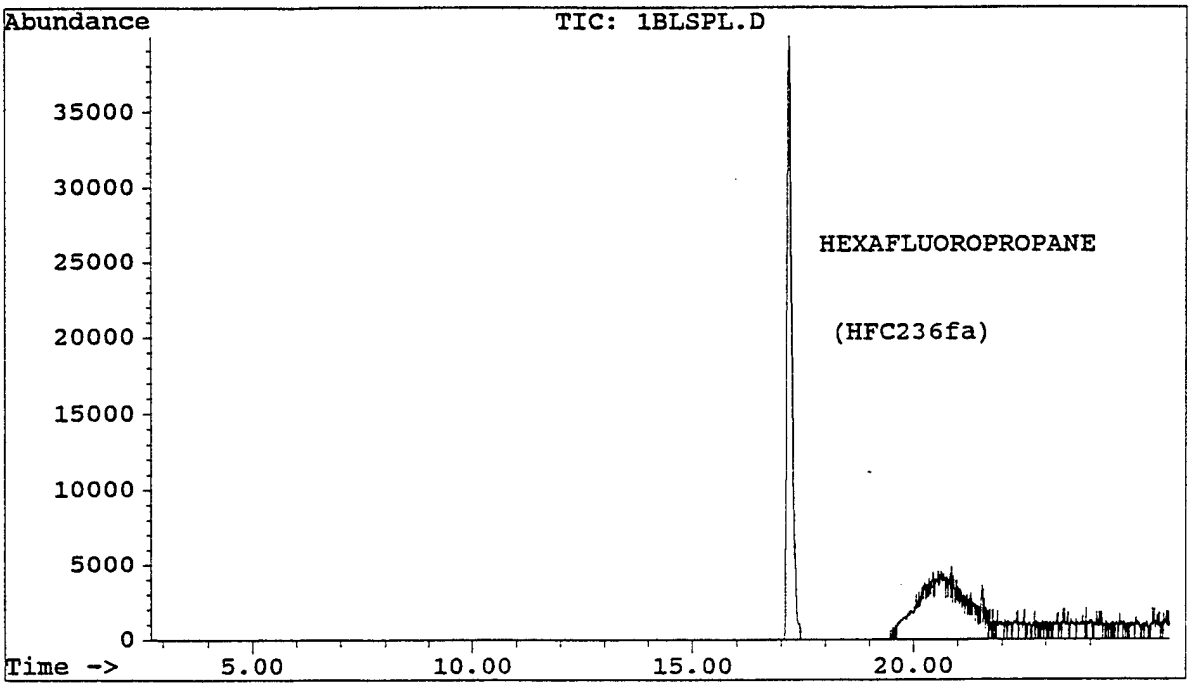
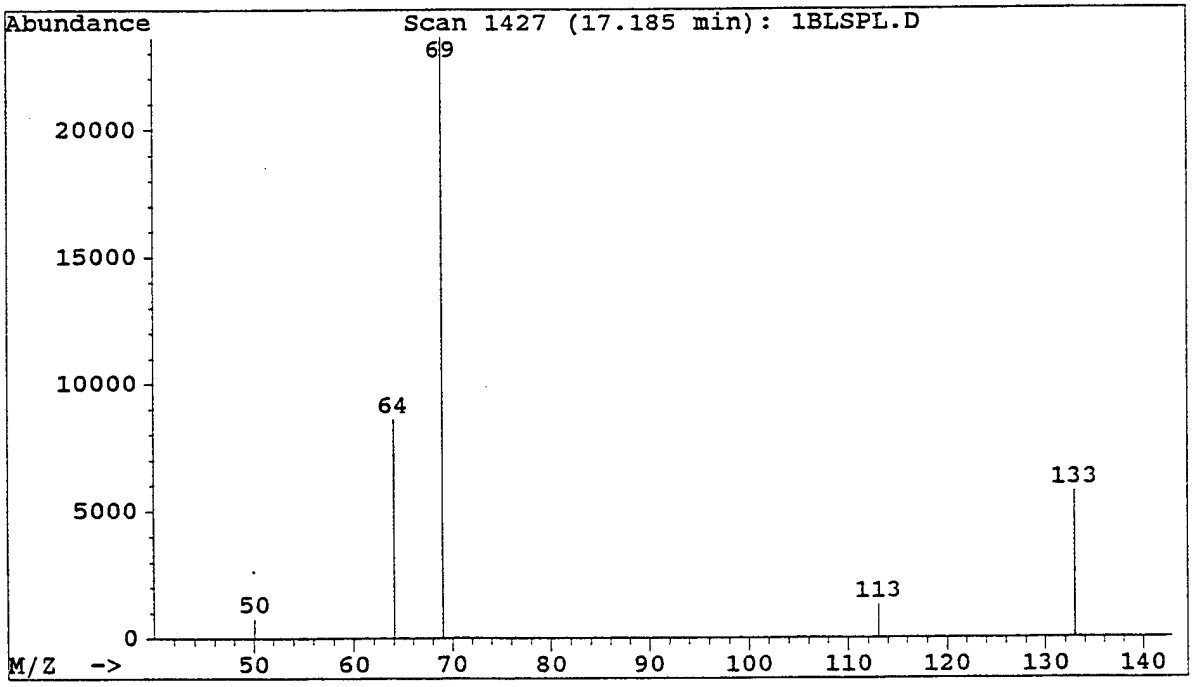


Figure 11. Chromatogram of Headspace Analysis of Blood Sample. The top graph is a chromatogram of the headspace analysis of a 0.5mL Blood sample with separation achieved using a Poraplot Q column, which is programmed at 45 °C - 10.0min/ 12 °C per min/ 175° C - 5.0min. The retention time of HFC-236fa is ~17.2 min. The lower graph is a mass spectrum of the peak at 17.2 min with major ions of 64, 69, 113, and 133.



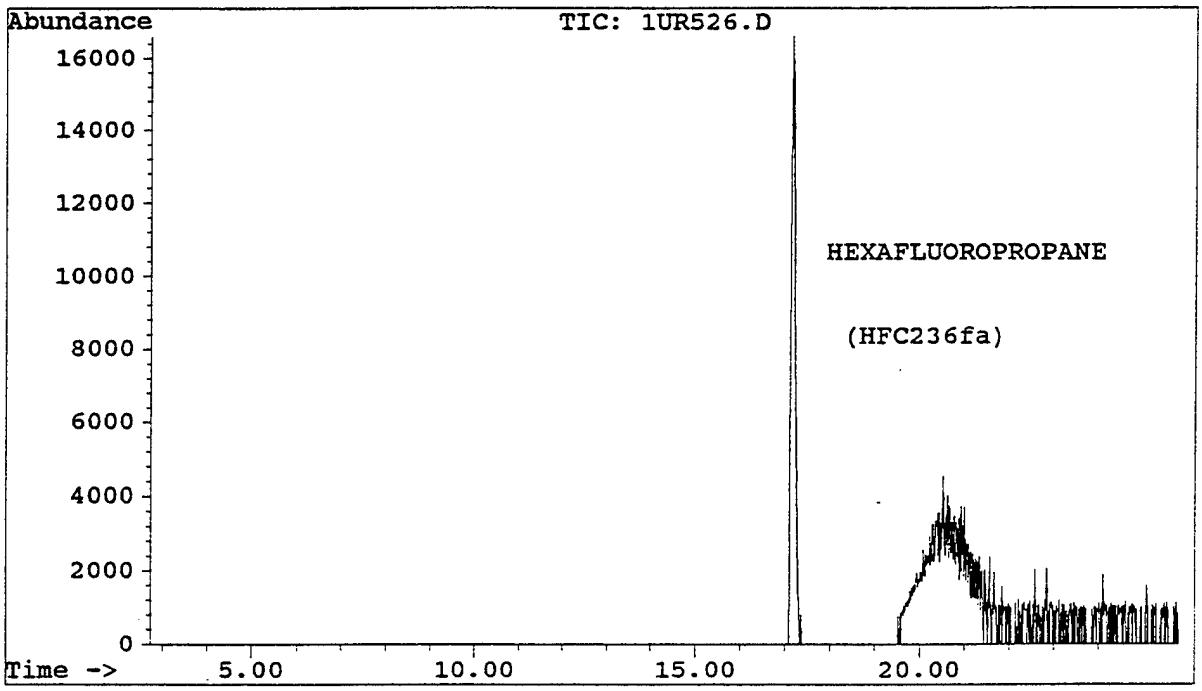
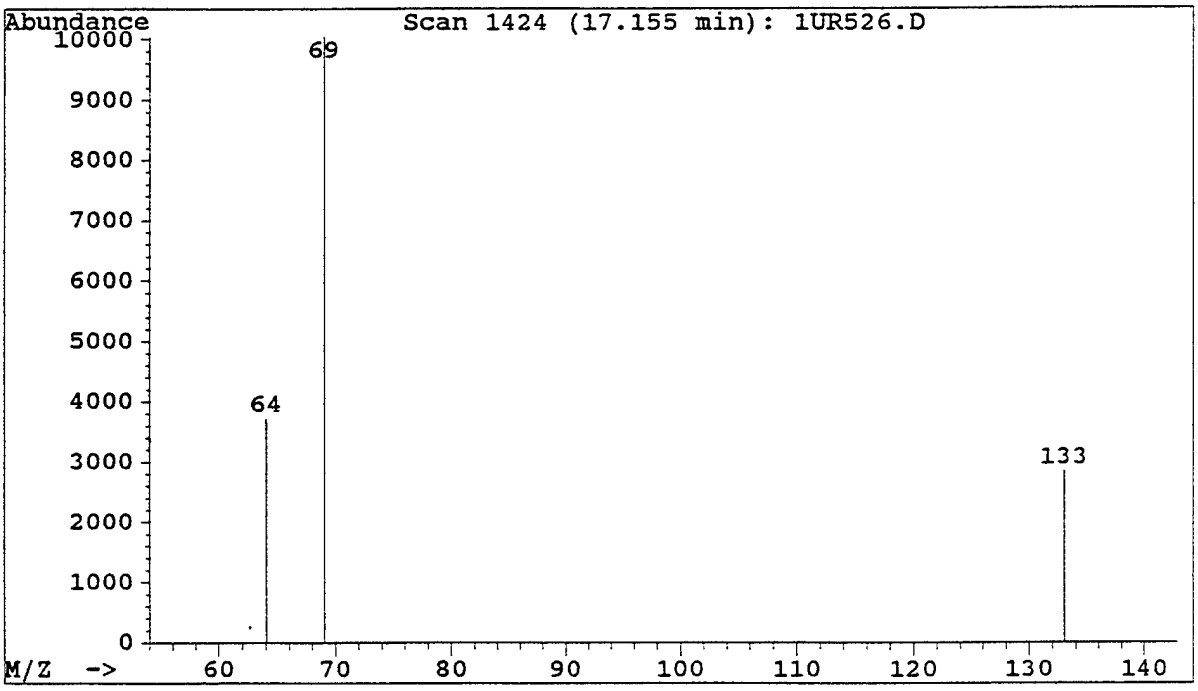


Figure 12. Chromatogram of Headspace Analysis of Urine Sample. The top graph is a chromatogram of the headspace analysis of a 0.5mL Urine sample with separation achieved using a Poraplot Q column, which is programmed at 45 °C - 10.0min/ 12 °C per min/ 175 °C - 5.0 min. The retention time of HFC-236fa is ~17.2 min. The lower graph is a mass spectrum of the peak at 17.2 min with major ions of 64, 69, 113, and 133.



SECTION 4
DISCUSSION

Gas Uptake Studies for Describing Metabolism and System Losses

This simulation approach for analysis of gas uptake data has been shown to distinguish between single and multiple metabolic pathways of several previously studied dihalomethanes and numerous other volatile organic compounds. In the case of HFC-236fa, the gas uptake results suggested a large uptake by the animals implying a high rate of metabolism for a chemical in which it is suspected there is little, if any, metabolism taking place.

The pattern of loss shown by the data in Figure 7 is inconsistent with the assumption of first-order or saturable metabolism. With first-order metabolism the slopes of the data lines should be parallel and with saturable metabolism the higher concentration data lines should approach the horizontal, at some intermediate concentration the slope should start curving downward and at lower concentrations the lines should be straight, parallel, and sloping downward. The data, as collected, show a random pattern of slope with concentration of exposure. Thus, the losses shown by the gas uptake data are assumed due to something other than metabolism. Furthermore, if metabolic constants were fitted to the data the rates of metabolism would be extremely high and not consistent with a chemical that is fluorinated at the end carbons, with the inability to find metabolites, and with the low tissue solubility of the chemical.

It was suspected that the humidity level in the chamber may play a role in the high loss rates observed during uptake determinations. During a live animal exposure, the humidity level is maintained at approximately 77 to 80% with the moisture trap in place. Under normal conditions for the loss runs, the chamber humidity level was equal to the laboratory humidity level. In order to test this theory the humidity level was increased in the chamber during loss runs by one of two methods.

The first method of increasing the chamber humidity level involved placing a beaker of water into the chamber during a loss run equal to the average weight of three rats, or approximately 550 grams. In addition, during an average exposure approximately 8 - 10 mL of water is collected in the moisture trap. Therefore eight milliliters of de-ionized water was placed into the moisture trap prior to addition of chemical into the chamber. A 20,000 ppm HFC-236fa loss run without animals was then completed. The humidity in the chamber averaged 64.64% ± 1.98% over the six-hour period and resulted in a loss rate of 0.30% per hour.

The second method of increasing the chamber humidity level included placing four Petri dishes containing 310 grams of de-ionized water into the chamber. Throughout the 20,000 ppm HFC-236fa loss run without animals, the chamber was warmed with a heating pad from under the

chamber. In addition, periodically throughout the loss run the chamber was warmed with a heat gun. The humidity in the chamber averaged 78.72% ± 6.29% over the six-hour exposure and resulted in a loss rate of 0.28% per hour. Neither of these two methods showed an increase in the percent loss of chemical per hour, thus rejecting the possibility of humidity levels influencing the loss of HFC-236fa concentration levels.

In addition, it was also suspected that the difference in loss rates was due to carbon dioxide that is added to the system during an exposure from the expired breath of the rats. However, a 500 ppm HFC-236fa loss run without dead rats was completed under the original system configuration, with supplemental carbon dioxide at a rate of 0.88 mL/g/h -- the same rate at which rats expire carbon dioxide. A loss rate of 1.50% per hour resulted. This experiment showed an increase in loss rate, although it did not increase the loss rate to the same rate at which it was being lost during a live animal exposure.

Metabolite Identification Studies

No metabolites of HFC-236fa were present at concentrations sufficiently high enough to be detected by the previously described methods. The detection limits for the esterified methyl trifluoroacetate was 0.49 μg/ml and methyl pentafluoroproprionate was 0.19 μg/ml. Approximate detection limits for the 1,3-difluoro-2-propanol was 0.3 μg/ml and for the 2,2,3,3,3-pentafluoro-1-propanol was 3.0 μg/ml. Further analyses involving extraction with other solvents, solvent-exchange, and volume reduction might be useful in identifying metabolites with low volatility. For compounds of high volatility, such as hexafluoroacetone, the headspace analysis is still probably the best approach. However, the results of the 26-May-95 exposure samples suggest that additional measures may need to be taken in handling these samples. In the future, no more than 0.5mL of blood or urine should be placed directly into a sealed analytical vial for headspace GC/MS in order to avoid possible losses of the parent compound or other highly volatile materials due to transferring the sample from one vial to another.

SECTION 5
CONCLUSIONS

1. The PBPK model could not adequately describe the disappearance of HFC-236fa from the chamber atmosphere during gas uptake experiments with the assumption that metabolism was the cause of chemical disappearance. The pattern of loss was, in fact, inconsistent with either first-order or saturable metabolism.

2. Possible cause of the unexplained loss may be associated with carbon dioxide exhalation during the exposure although final confirmation would require further investigation.

3. HFC-236fa had low solubility (partition) in blood and tissues and had minimal, if any, enzymatic metabolism in rats.

4. Explicit analyses for potential metabolites in blood, urine, and feces failed to detect anthing but HFC-236fa itself.

SECTION 6

REFERENCES

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Andersen, M.E., H.J. Clewell, M.L. Gargas, F.A. Smith, and R.H. Reitz. 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87:185-205.

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McLafferty, F.W. 1980. *The Interpretation of Mass Spectra*, 3rd Edition.

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APPENDIX A

CODES AND COMMAND FILE FOR COMPUTER SIMULATION OF HFC-236fa PHARMACOKINETICS

```
PROGRAM: CLOSED CHAMBER MODEL HFC-236fa  GAS-UPTAKE EXPOSURES
'Based on:'
'Template Model with Code for Gut and Liver - 30 March 1993'
'-------------------------------------------------------'
INTEGER  J
ARRAY CONCJ(6), BWJ(6), KLJ(6)
CONSTANT CONCJ = 100.0,530.0,7300.0,12600.0,18000.0,24500.0
CONSTANT BWJ =.257,.254,.242,.237,.197,.237
CONSTANT KLJ=.02,.007,.004,.004,.004,.004
CONSTANT J=1, JJ=1.0

INITIAL

ALGORITHM IALG = 2  $'Gear method for stiff systems'

    'Timing commands'

CONSTANT   TSTOP = 6.   $'Length of experiment (hrs)'
CONSTANT    CINT = .1   $'Communication interval'

'CONSTANT   KL  = .004 '  $'FIRST ORDER CHAMBER LOSS'
'CONSTANT   BW = 0.23 '  $'Body weight (kg)'

J = INT(JJ)
CONC = CONCJ(J)
BW = BWJ(J)
KL=KLJ(J)

CONSTANT   QPC = 14.00   $'Alveolar ventilation rate (l/hr)'
CONSTANT   QCC = 14.00   $'Cardiac output (l/hr)'

CONSTANT   QLC = .032  $'Fractional blood flow to liver'
CONSTANT   QGC = .183  $'Fractional blood flow to gut'
CONSTANT   QFC = .058  $'Fractional blood flow to fat'
CONSTANT   QSC = .255  $'Fractional blood flow to slow'
CONSTANT   QRC = .472  $'Fractional blood flow to rapid'

CONSTANT   VLC = .037  $'Fraction liver tissue'
CONSTANT   VGC = .033  $'Fraction gut tissue'
CONSTANT   VSC = .558  $'Fraction slow tissue'
CONSTANT   VRC = .031  $'Fraction rapid tissue'

VFC = .01*(35.0*BW+2.1)  $'Fraction fat tissue'
```

```
CONSTANT   PLA = 0.564   $'Liver/air partition coefficient'
CONSTANT   PGA = 0.564   $'Gut/air partition coefficient'
CONSTANT   PFA = 3.689   $'Fat/air partition coefficient'
CONSTANT   PSA = 0.868   $'Slowly perfused tissue/air partition'
CONSTANT   PRA = 0.564   $'Richly perfused tissue/air partition'
CONSTANT   PB =  0.493   $'Blood/air partition coefficient'

PL=PLA/PB  $'Liver/blood partition coefficient'
PG=PGA/PB  $'Gut/blood partition coefficient'
PF=PFA/PB  $'Fat/blood partition coefficient'
PS=PSA/PB  $'Slow/blood partition coefficient'
PR=PRA/PB  $'Rich/blood partition coefficient'

CONSTANT   MW = 152.0  $'Molecular weight (g/mol)'
CONSTANT   VMAXC=0.0 $'Maximum velocity of metabolism (mg/hr-1kg)'
CONSTANT   KM = 10000.    $'Michaelis-Menten constant (mg/l)'
CONSTANT   KFC = 0.   $'First order metabolism rate constant (/hr-1kg)'
CONSTANT   CONC=100.   $'Inhaled concentration (ppm)'
CONSTANT   RATS = 3.   $'Number of rats (for closed chamber)'
CONSTANT   VCHC = 8.0  $'Volume of closed chamber (l)'
CONSTANT   SODA = .075  $'Volume of soda lime (l)'

VCH = VCHC-(RATS*BW)-SODA   $'Net chamber volume (l)'
AI0 = CONC*VCH*MW/24450.    $'Initial amount in chamber (mg)'

    'Scaled parameters'

      QC = QCC*BW**0.75
      QP = QPC*BW**0.75
      QL = QLC*QC
      QG = QGC*QC
      QF = QFC*QC
      QS = QSC*QC
      QR = QRC*QC
      VL = VLC*BW
      VG = VGC*BW
      VF = VFC*BW
      VS = VSC*BW
      VR = VRC*BW
      VMAX = VMAXC*BW**0.75
      KF = KFC/BW**0.25
      VK = VMAXC/KM

END     $'End of initial'

DYNAMIC

DERIVATIVE
```

```
      'CI = Concentration in inhaled air (mg/l)'
      RAI = RATS*QP*(CA/PB-CI)-(KL*AI)
       AI = INTEG(RAI,AI0)                    $ 'CHAMBER'
       CI = AI/VCH                          $ 'WITH X RATS'
       CP = CI*24450./MW
'-------------------------------------'
      'CA = Concentration in arterial blood (mg/l)'
       CA = (QC*CV+QP*CI)/(QC+(QP/PB))

      'AX = Amount exhaled per rat (mg)'
       CX = CA/PB
    CXPPM = (0.7*CX+0.3*CI)*24450./MW
      RAX = QP*CX
       AX = INTEG(RAX,0.)

      'AS = Amount in slowly perfused tissues per rat (mg)'
      RAS = QS*(CA-CVS)
       AS = INTEG(RAS,0.)
      CVS = AS/(VS*PS)
       CS = AS/VS

      'AR = Amount in rapidly perfused tissues per rat (mg)'
      RAR = QR*(CA-CVR)
       AR = INTEG(RAR,0.)
      CVR = AR/(VR*PR)
       CR = AR/VR

      'AF = Amount in fat tissue per rat (mg)'
      RAF = QF*(CA-CVF)
       AF = INTEG(RAF,0.)
      CVF = AF/(VF*PF)
       CF = AF/VF

      'AG = Amount in gut tissue per rat (mg)'
      RAG = QG*(CA-CVG)
       AG = INTEG(RAG,0.)
      CVG = AG/(VG*PG)
       CG = AG/VG

      'AL = Amount in liver tissue per rat (mg)'
      RAL = QL*(CA-CVL)+QG*(CVG-CVL)-RAM
       AL = INTEG(RAL,0.)
      CVL = AL/(VL*PL)
       CL = AL/VL

      'AM = Amount metabolized per rat  (mg)'
      RAM = (VMAX*CVL)/(KM+CVL) + KF*CVL*VL  $'(mg/hr)'
       AM = INTEG(RAM,0.)                    $'Amount (mg)'
```

```
      'CV = Mixed venous blood concentration per rat (mg/l)'
      CV = (QF*CVF + (QL+QG)*CVL + QS*CVS + QR*CVR)/QC

'AMOUNT INHALED PER RAT'
      RINH = QP*CI
      AINH = INTEG(RINH,0)

'TMASS = MASS BALANCE PER RAT'
      TMASS = (AS+AR+AF+AM+AL+AX+AG)
      BAL = AINH - TMASS

'AMOUNT IN RAT'
      AINRAT=AINH-AX


TERMT (T.GE.TSTOP)

END       $'End of derivative'
END       $'End of dynamic'
END       $'End of program'
```

```
'UPTK236.CMD'
'GAS UPTAKE DATA FOR HFC-236Fa'

SET TITLE = 'HFC-236fa Gas Uptake'

PREPAR T,'ALL'

SET GRDCPL=.F.    $'Turns off grid lines'

PROCED ARRAY1
SET CONCJ=100.0,530.0,7300.0,12600.0,18000.0,24500.0
SET BWJ=.257,.254,.242,.237,.197,.237
SET KLJ=.02,.0067,.004,.004,.004,.004
SET J=1,JJ=1.0
END

PROCED ARRAY2
SET CONCJ=100.0,530.0,2350.0,7300.0,18000.0
SET BWJ=.257,.254,.237,.242,.197
SET KLJ=.02,.0067,.004,.004,.004
SET J=1,JJ=1.0
END


PROCED HFC236
SET KFC=0.0,KM=10000.,VMAXC=0.0
SET PLA=.564, PGA=.564, PFA=3.689, PRA=.564
SET PSA=.868, PB=.493
SET MW=152.
SET RATS=3, VCHC=8., SODA=.075
SET QPC=14.0, QCC=14.0
DISPLAY QPC,QCC,VMAXC,KM,KFC,PB,PLA,PGA,PFA,PSA
END

PROCED INHAL
ARRAY1
DATA
T      CP          JJ
0.0    .       1.0  INITIAL
0.08333        98.56  .
0.16667        97.13  .
0.25   96.21   .
0.33333        95.32  .
0.41667        94.21  .
0.5    93.48   .
0.75   90.69   .
1.     88.23   .
1.25   85.70   .
```

1.5 83.55 .
1.75 80.95 .
2. 78.70 .
2.25 76.74 .
2.5 73.95 .
2.75 71.19 .
3. 68.61 .
3.25 66.22 .
3.5 63.98 .
3.75 62.02 .
4. 60.06 .
4.25 58.31 .
4.5 56.50 .
4.75 54.80 .
5. 53.24 .
5.25 51.80 .
5.5 50.40 .
5.75 48.92 .
6. 47.61 .
0.0 . 2.0 INITIAL
0.08333 524.57 .
0.1667 519.49 .
0.25 514.70 .
0.33333 511.00 .
0.42667 507.10 .
0.5 502.72 .
0.75 492.12 .
1. 480.76 .
1.25 468.40 .
1.50 458.08 .
1.75 444.60 .
2.00 431.47 .
2.25 419.94 .
2.50 406.75 .
2.75 393.26 .
3.00 377.45 .
3.25 362.50 .
3.50 349.79 .
3.75 337.47 .
4.00 327.63 .
4.25 317.42 .
4.50 307.61 .
4.75 299.42 .
5.00 290.88 .
5.25 283.08 .
5.50 275.90 .
5.75 268.56 .
6.00 263.13 .
0.0 . 3.0 INITIAL

0.08333 7282.41 .
0.1667 7184.50 .
0.25 7099.59 .
0.33333 7058.74 .
0.42667 6978.57 .
0.5 6898.06 .
0.75 6635.63 .
1. 6428.39 .
1.25 6315.36 .
1.50 6222.96 .
1.75 6124.85 .
2.00 6035.99 .
2.25 5897.17 .
2.50 5785.43 .
2.75 5614.36 .
3.00 5454.76 .
3.25 5310.53 .
3.50 5139.78 .
3.75 4989.83 .
4.00 4861.20 .
4.25 4707.67 .
4.50 4548.91 .
4.75 4406.51 .
5.00 4208.78 .
5.25 4113.84 .
5.50 4000.81 .
5.75 3976.19
6.00 3879.58 .
0.0 . 4.0 INITIAL
0.08333 12499.27 .
0.1667 12308.40 .
0.25 12255.38 .
0.33333 12086.51 .
0.42667 12026.22 .
0.5 11905.06 .
0.75 11626.46 .
1. . .
1.25 10859.77 .
1.50 10574.11 .
1.75 10193.83 .
2.00 9256.65 .
2.25 9092.24 .
2.50 8889.37 .
2.75 8620.25 .
3.00 8438.97 .
3.25 8293.83 .
3.50 8164.83 .
3.75 8011.81 .
4.00 7937.95 .

4.25 7817.46 .
4.50 7762.59 .
4.75 7619.75 .
5.00 7482.62 .
5.25 7348.01 .
5.50 7130.70 .
5.75 6982.10 .
6.00 6860.25 .
0.0 . 5.0 INITIAL
0.08333 17820.60
0.1667 17903.84
0.25 17734.69
0.33333 17609.19
0.42667 17638.94
0.5 17487.27
0.75 17108.35
1. 16961.01
1.25 16711.91
1.5 16543.37
1.75 16449.43
2.0 16315.02
2.25 16030.81
2.5 16068.86
2.75 15987.73
3.0 15933.20
3.25 15765.62
3.5 15637.38
3.75 15578.43
4.0 15322.06
4.25 15064.96
4.5 14881.98
4.75 14439.79
5.0 14286.90
5.25 13983.57
5.5 13736.30
5.75 13600.76
6.0 13242.08
0.0 . 6.0 INITIAL
0.08333 24463.48 .
0.1667 23904.31 .
0.25 23727.66 .
0.33333 24068.60 .
0.42667 22873.35 .
0.5 23562.12 .
0.75 21390.90 .
1. 20736.12 .
1.25 20474.45 .
1.50 19969.24 .
1.75 19751.64 .

```
2.00    19280.49    .
2.25    19065.29    .
2.50    18857.92    .
2.75    18556.32    .
3.00    18191.79    .
3.25    17741.26    .
3.50    17377.27    .
3.75    17080.33    .
4.00    16612.52    .
4.25    16316.68    .
4.50    15977.99    .
4.75    15632.67    .
5.00    15240.43    .
5.25    15049.64    .
5.50    14846.62    .
5.75    14552.58    .
6.00    14439.32    .
END
END
```

```
PROCED INHAL2
ARRAY2
DATA
T       CP          JJ
0.0     .       1.0 INITIAL
0.08333         98.56   .
0.16667         97.13   .
0.25    96.21   .
0.33333         95.32   .
0.41667         94.21   .
0.5     93.48   .
0.75    90.69   .
1.      88.23   .
1.25    85.70   .
1.5     83.55   .
1.75    80.95   .
2.      78.70   .
2.25    76.74   .
2.5     73.95   .
2.75    71.19   .
3.      68.61   .
3.25    66.22   .
3.5     63.98   .
3.75    62.02   .
4.      60.06   .
4.25    58.31   .
4.5     56.50   .
4.75    54.80   .
```

5. 53.24 .
5.25 51.80 .
5.5 50.40 .
5.75 48.92 .
6. 47.61 .
0.0 . 2.0 INITIAL
0.08333 524.57 .
0.1667 519.49 .
0.25 514.70 .
0.33333 511.00 .
0.42667 507.10 .
0.5 502.72 .
0.75 492.12 .
1. 480.76 .
1.25 468.40 .
1.50 458.08 .
1.75 444.60 .
2.00 431.47 .
2.25 419.94 .
2.50 406.75 .
2.75 393.26 .
3.00 377.45 .
3.25 362.50 .
3.50 349.79 .
3.75 337.47 .
4.00 327.63 .
4.25 317.42 .
4.50 307.61 .
4.75 299.42 .
5.00 290.88 .
5.25 283.08 .
5.50 275.90 .
5.75 268.56 .
6.00 263.13 .
0.0 . 3.0 INITIAL
0.08333 2335.06 .
0.1667 2319.08 .
0.25 2306.89 .
0.33333 2285.19 .
0.42667 2266.27 .
0.5 2243.54 .
0.75 2208.63 .
1. 2161.61 .
1.25 2113.25 .
1.5 2073.83 .
1.75 2026.53 .
2. 1982.49 .
2.25 1942.25 .
2.5 1903.28 .

2.75 1869.41 .
3. 1839.06 .
3.25 1804.10 .
3.5 1773.48 .
3.75 1742.87 .
4. 1709.76 .
4.25 1675.65 .
4.5 1643.48 .
4.75 1611.12 .
5. 1586.28 .
5.25 1561.41 .
5.5 1539.30 .
5.75 1512.99 .
6. 1494.42 .
0.0 . 4.0 INITIAL
0.08333 7282.41 .
0.1667 7184.50 .
0.25 7099.59 .
0.33333 7058.74 .
0.42667 6978.57 .
0.5 6898.06 .
0.75 6635.63 .
1. 6428.39 .
1.25 6315.36 .
1.50 6222.96 .
1.75 6124.85 .
2.00 6035.99 .
2.25 5897.17 .
2.50 5785.43 .
2.75 5614.36 .
3.00 5454.76 .
3.25 5310.53 .
3.50 5139.78 .
3.75 4989.83 .
4.00 4861.20 .
4.25 4707.67 .
4.50 4548.91 .
4.75 4406.51 .
5.00 4208.78 .
5.25 4113.84 .
5.50 4000.81 .
5.75 3976.19 .
6.00 3879.58 .
0.0 . 5.0 INITIAL
0.08333 17820.60 .
0.1667 17903.84 .
0.25 17734.69 .
0.33333 17609.19 .
0.42667 17638.94 .

```
0.5     17487.27    .
0.75    17108.35    .
1.      16961.01    .
1.25    16711.91    .
1.5     16543.37    .
1.75    16449.43    .
2.0     16315.02    .
2.25    16030.81    .
2.5     16068.86    .
2.75    15987.73    .
3.0     15933.20    .
3.25    15765.62    .
3.5     15637.38    .
3.75    15578.43    .
4.0     15322.06    .
4.25    15064.96    .
4.5     14881.98    .
4.75    14439.79    .
5.0     14286.90    .
5.25    13983.57    .
5.5     13736.30    .
5.75    13600.76    .
6.0     13242.08    .
END
END
```