

Human Safety and Pharmacokinetics of the CFC Alternative Propellants HFC 134a (1,1,1,2-Tetrafluoroethane) and HFC 227 (1,1,1,2,3,3,3-Heptafluoropropane) Following Whole-Body Exposure

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HFC 134a (1,1,1,2-tetrafluoroethane) and HFC 227 (1,1,1,2,3,3,3-heptafluoropropane) are used to replace chlorofluorocarbons (CFCs) in refrigerant and aerosol applications, including medical use in metered-dose inhalers. Production and consumption of CFCs are being phased out under the Montreal Protocol on Substances that Deplete the Ozone Layer. The safety and pharmacokinetics of HFC 134a and HFC 227 were assessed in two separate double-blind studies. Each HFC (hydrofluorocarbon) was administered via whole-body exposure as a vapor to eight (four male and four female) healthy volunteers. Volunteers were exposed, once weekly for 1 h, first to air and then to ascending concentrations of HFC (1000, 2000, 4000, and 8000 parts per million (ppm)), interspersed with a second air exposure and two CFC 12 (dichlorodifluoromethane) exposures (1000 and 4000 ppm). Comparison of either HFC 134a or HFC 227 to CFC 12 or air gave no clinically significant results for any of the measured laboratory parameters. There were no notable adverse events, there was no evidence of effects on the central nervous system, and there were no symptoms of upper respiratory tract irritation. HFC 134a, HFC 227, and CFC 12 blood concentrations increased rapidly and in an exposure-concentration-dependent manner, although not strictly proportionally, and approached steady state. Maximum blood concentrations (C_{\max}) tended to be higher in males than females; in the HFC 227 study, these were statistically significantly ($P < 0.05$) higher in males for each HFC 227 and CFC 12 exposure level. In the HFC 134a study, the gender difference in C_{\max} was only statistically significant ($P < 0.05$) for CFC 12 at 4000 ppm and HFC 134a at 8000 ppm. Following the end of exposure, blood concentrations declined rapidly, predominantly biphasically and independent of exposure concentration. For the HFC 134a study, the $t_{1/2\alpha}$ (α elimination half-life) was short for both CFC 12 and HFC 134a (< 11 min). The $t_{1/2\beta}$ (β elimination half-life) across all exposure concentrations was a mean of 36 and 42 min for CFC 12 and HFC

134a, respectively. Mean residence time (MRT) was an overall mean of 42 and 44 min for CFC 12 and HFC 134a, respectively. In the HFC 227 study, $t_{1/2\alpha}$ for both CFC 12 and HFC 227, at each exposure level, was short (< 9 min) and tended to be lower in males than females. For CFC 12 mean $t_{1/2\beta}$ ranged from 23 to 43 min and for HFC 227 the mean range was 19–92 min. The values tended to be lower for females than males for HFC 227. For both CFC 12 and HFC 227, MRT was statistically significantly lower ($P < 0.05$) in males than females and independent of exposure concentration. For CFC 12, MRT was a mean of 37 and 45 min for males and females, respectively, and for HFC 227 MRT was a mean of 36 and 42 min, respectively. Exposure of healthy volunteers to exposure levels up to 8000 ppm HFC 134a, 8000 ppm HFC 227, and 4000 ppm CFC 12 did not result in any adverse effects on pulse, blood pressure, electrocardiogram, or lung function. © 2000 Academic Press

INTRODUCTION

For many years, chlorofluorocarbons (CFCs)¹ have been used in such diverse applications as refrigerants,

¹ Abbreviations used: AUC_{0-t} , area under the concentration–time curve from start of exposure to the last measured concentration time point; $AUC_{0-\infty}$, area under the concentration–time curve from start of exposure to infinity; CFCs, chlorofluorocarbons; CFC 12, dichlorodifluoromethane; C_{\max} , maximum blood concentration; ECETOC, European Centre for Ecotoxicity and Toxicology of Chemicals; ECG, electrocardiogram; halon 1301, bromotrifluoromethane; HFC, hydrofluorocarbon; HFC 134, 1,1,1,2-tetrafluoroethane; HFC 134a, 1,1,1,2-tetrafluoroethane; HFC 227, 1,1,1,2,3,3,3-heptafluoropropane; IPACT-I, International Pharmaceutical Aerosol Consortium for Toxicology Testing of HFA-134a; IPACT-II, International Pharmaceutical Aerosol Consortium for Toxicology Testing of HFA-227; MDIs, metered-dose inhalers; MRT, mean residence time from start of exposure to the last measured concentration time point; PEF, peak expiratory flow; ppm, parts per million; SD, standard deviation; $t_{1/2\alpha}$, α elimination half-life; $t_{1/2\beta}$, β elimination half-life; T_{\max} , time to maximum blood concentration; UNEP, United Nations Environmental Program.

propellants for personal care items and medicinal aerosols, and foam blowing agents and in precision cleaning. Their widespread popularity stemmed from their low toxicity, stability, and efficacy. However, Molina and Roland (1974) suggested that these stable CFCs could be transported to the stratosphere, where they would break down to release chlorine radicals, which could catalyze breakdown of stratospheric ozone. This led to research to find suitable replacements. The program was intensified with the ratification of the 1987 Montreal Protocol and its amendments (United Nations Environmental Program, 1991) calling for a phaseout of the CFCs.

HFC 134a (1,1,1,2-tetrafluoroethane) and HFC 227 (1,1,1,2,3,3,3-heptafluoropropane) are used as CFC replacements in refrigeration and aerosols, including medically in metered-dose inhalers (MDIs). Because these molecules have no chlorine, they do not contribute to the breakdown of stratospheric ozone. As HFC 134a and HFC 227 are being used in a wide variety of applications, their toxicity has been rigorously evaluated in many studies (Collins *et al.*, 1995; Alexander *et al.*, 1995; Alexander and Libretto, 1995; European Centre for Ecotoxicity and Toxicology of Chemicals (ECETOC), 1995; International Pharmaceutical Aerosol Consortium for Toxicology Testing of HFC 227 (IPACT-II), unpublished studies; Skaggs *et al.*, 1995). HFC 134a and HFC 227 have been shown to have low acute, subchronic, and chronic toxicity.

Exposures of rats to up to 50,000 parts per million (ppm) HFC 134a, 6 h per day, 5 days per week for 2 years did not exhibit significant signs of toxicity. In the 2-year study, an increase in Leydig cell tumors was seen in rats exposed to HFC 134a at 50,000 ppm, 6 h per day. The no-observed-effect level was 10,000 ppm. This tumor is common in rats but rare in humans and involves different endocrine pathways. Thus, the Leydig tumor in rats has little significance in humans (Cook *et al.*, 1999). Exposures of HFC 227 to rats of up to 240,000 ppm, 1 h daily, for 2 years did not result in significant signs of toxicity. The no-observed-effect level was at least 240,000 ppm.

The threshold for cardiac sensitization in dogs was 75,000 ppm for HFC 134a (ECETOC, 1995) and 100,000 ppm for HFC 227 (IPACT-II, unpublished data). HFC 134a and HFC 227 were not developmental or reproductive toxins in inhalation studies that used exposure levels as high as 40,000 ppm HFC 134a and 150,000 ppm HFC 227 in rabbits and 300,000 ppm HFC 134a and 150,000 ppm HFC 227 in rats. Neither HFC 134a nor HFC 227 were active in a series of genetic assays (Collins *et al.*, 1995; IPACT-II, unpublished data).

HFC 134a and HFC 227 have been separately evaluated as replacement propellants for MDIs (Dalby *et al.*, 1990). Clinical trials (Harrison *et al.*, 1996; Alexander, 1995; Blumenthal *et al.*, 1997; IPACT-II, unpublished

data; Hermann *et al.*, 1998) have formed a part of this evaluation. These studies showed no adverse events following multiple exposures of the volume normally dispensed by the MDI. A recent study designed to measure human blood levels of HFC 134a and HFC 227 resulting from short-term exposures (Vinegar *et al.*, 1997) was undertaken to validate a pharmacokinetic model. The model was to be used for risk assessment for short, high-level exposures such as might be encountered through the use of HFC 134a or HFC 227 as a flame-suppressant agent. The study involved serial blood sampling, via an indwelling cannula, while subjects inhaled HFC 134a or HFC 227 through a one-way mask. The exposure levels were 4000 ppm for HFC 134a and 6400 ppm for HFC 227. Although the control exposures with halon 1301 (bromotrifluoromethane) at 5000 ppm for 30 min were without incident, the first subject exposed to HFC 134a exhibited a rapid drop in pulse and blood pressure and fainted, and the second subject exhibited an increase in blood pressure and heart rate about 10 min after initiation of the exposure. The first subject exposed to HFC 227 exhibited a rapid rise in pulse rate within minutes of the start of exposure. The exposures to the HFCs were discontinued at this point. As the two HFC 134a observations appeared in conflict with one another and all of the observations were completely unexpected based on clinical trial and animal experimental data, the studies described herein were commissioned.

The two studies reported in this publication, conducted at TNO, were commissioned by several groups to study HFC 134a and HFC 227 using state-of-the-art toxicological methodology. The purpose of the studies was to provide data on the effects of exposure to vapors of either HFC 134a or HFC 227 under carefully controlled conditions and thus determine if the effects described by Vinegar *et al.* (1997) could be attributed to HFC 134a or HFC 227 exposure.

MATERIAL AND METHODS

Material

HFC 134a manufactured by E. I. DuPont (Wilmington, DE), HFC 227 manufactured by Solvay Fluor and Derivate (Frankfurt, Germany), and CFC 12 manufactured by ICI Klea (Cheshire, England) supplied in pressurized gas cylinders were used to generate test atmospheres. HFC 134a was 99.92% pure and both HFC 227 and CFC 12 were 99.99% pure (supplier information). Air passed through activated carbon (filter type 2000 RB/C, Camfil BV, Ede, The Netherlands) and dust filters (filter type T 15/250, ACS Filtertechnik BV, Alkmaar, The Netherlands) was used.

Study Population

Eight healthy nonsmoking volunteers, four males and four females, aged between 20 and 24 years, with

normal electrocardiogram (ECG), lung function (peak expiratory flow (PEF) >80% predicted normal), body fat volume (<30%), and normal clinical laboratory parameters were enrolled for each study. Volunteers were excluded if they were taking chronic medication, were claustrophobic, or had an alcohol intake of more than 21 units/week. Volunteers were recruited to take part in only one of the two studies. Women were excluded if pregnant or lactating and were tested for pregnancy prior to enrollment and prior to each exposure. All volunteers gave their written informed consent. The studies were approved by the local ethics committee and were carried out in accordance with the provisions of the Declaration of Helsinki.

Study Design

The studies were within-subject design that compared rising exposure concentrations of HFC 134a versus CFC 12 and air in one study and HFC 227 versus CFC 12 and air in the other. While starting at a lower exposure level (1000 ppm) than in the study described by Vinegar *et al.* (1997), these studies were designed to include an exposure level (8000 ppm) higher than in the earlier study and exposure was for a much longer time, 1 h. Volunteers were individually exposed in a whole-body exposure chamber.

Exposure was for 1 h on eight separate occasions, separated generally by 7 days; two air exposures, two CFC 12 exposures (at 1000 and 4000 ppm), and four exposures (at 1000, 2000, 4000, and 8000 ppm) of either HFC 134a or HFC 227. On one occasion, for one volunteer in the HFC 134a study, the interval between exposures was 14 days. For one volunteer in the HFC 227 study, the first exposure to air had to be repeated the following week (the Holter failed during the original exposure) and the next exposure (to 4000 ppm CFC 12) was performed 2 days later. Subsequent exposures were according to schedule up to exposure 5. The subject then became ill (not treatment related) and exposures 6, 7, and 8 were postponed by 1 week. For a second subject in the HFC 227 study, exposure session 4 (HFC 227, 4000 ppm) had to be repeated (the Holter failed during the original exposure) and subsequent exposure sessions were postponed by 1 week. Volunteers were assigned to an exposure schedule such that males and females were exposed equally over morning and afternoon sessions.

The air-conditioned exposure chamber was constructed from polyurethane and stainless-steel panels (volume 13.6 m³, flow rate approximately 500 L/min) and was operated at 22–25°C and 40–60% relative humidity. Generation of the test atmosphere was accomplished by passing liquid propellant through an evaporator (type DGM 5853 GPL, Renzo Landi, Reggio Emilia, Italy) which was heated to 40°C with water. Materials used in the making of the evaporator include

aluminum, elastomere (rubber), and brass. The resulting vapor was introduced in the main air supply of the exposure chamber via a calibrated rotameter. The chamber concentration was sampled at a single point and measured for 1 min, at an interval of 2 min (infrared absorption using a photoacoustic method, dual-gas monitor type 3425, Bruel & Kjaer, Naerum, Denmark). Prestudy validation work had shown the chamber exposure levels were homogenous and stable. A sample of the exposure chamber atmosphere was taken for gas chromatography analysis to confirm propellant identity. Chamber oxygen levels were also measured (Thermox, Thermox, Pittsburgh, PA). Volunteers entered the chamber, via an air lock, after target concentrations had been established. During entry into the chamber exposure concentrations declined somewhat. To compensate for this dip, the input flow rate was increased slightly for a short time. While in the exposure chamber, the volunteer was comfortably seated with an arm through a glove port into a "box" with access from both inside and outside the chamber. The access in and out of the box allowed blood samples to be taken during exposure.

Assessment Procedures

On each exposure day, the following procedures were performed:

- ECG was continuously recorded from between 15 and 45 min prior to exposure to 30 min postexposure (60 min following final exposure). ECG was recorded by online telemetry using a Holter system and analyzed offline by Stichting Fysiologic (Foundations Fysiologic, Zeist, Holland).
- Blood pressure and pulse rate were measured upon arrival to the unit, 10 min prior to exposure, 10 min after start of exposure, and thereafter at approximately 10-min intervals until 30 min postexposure. Blood pressure was measured using an automatically inflating and deflating system (Boso Oscillomat, Bosch + Sohn GmbH, Germany); pulse rate was recorded simultaneously.
- Lung function (as PEF) was measured using a SensorLab spirometer (SensorMedics BV, Bithoven, The Netherlands) at 45 min prior to exposure and 75 min after the end of exposure. Three attempts were recorded and expressed as percentage of predicted normal value. The best of three attempts is reported.
- Serial blood samples (3 ml) were drawn via an indwelling cannula (Abbott BV, Amstelveen, The Netherlands) 20 min prior to exposure, 1, 3, 5, 15, 29, and 55 min during exposure and 2, 5, 10, 20, 30, 40, and 60 min following the end of exposure. Twenty-four hours after the final exposure, a physical examination was conducted, blood was taken for clinical chemistry and hematology, a urine sample was obtained for analysis,

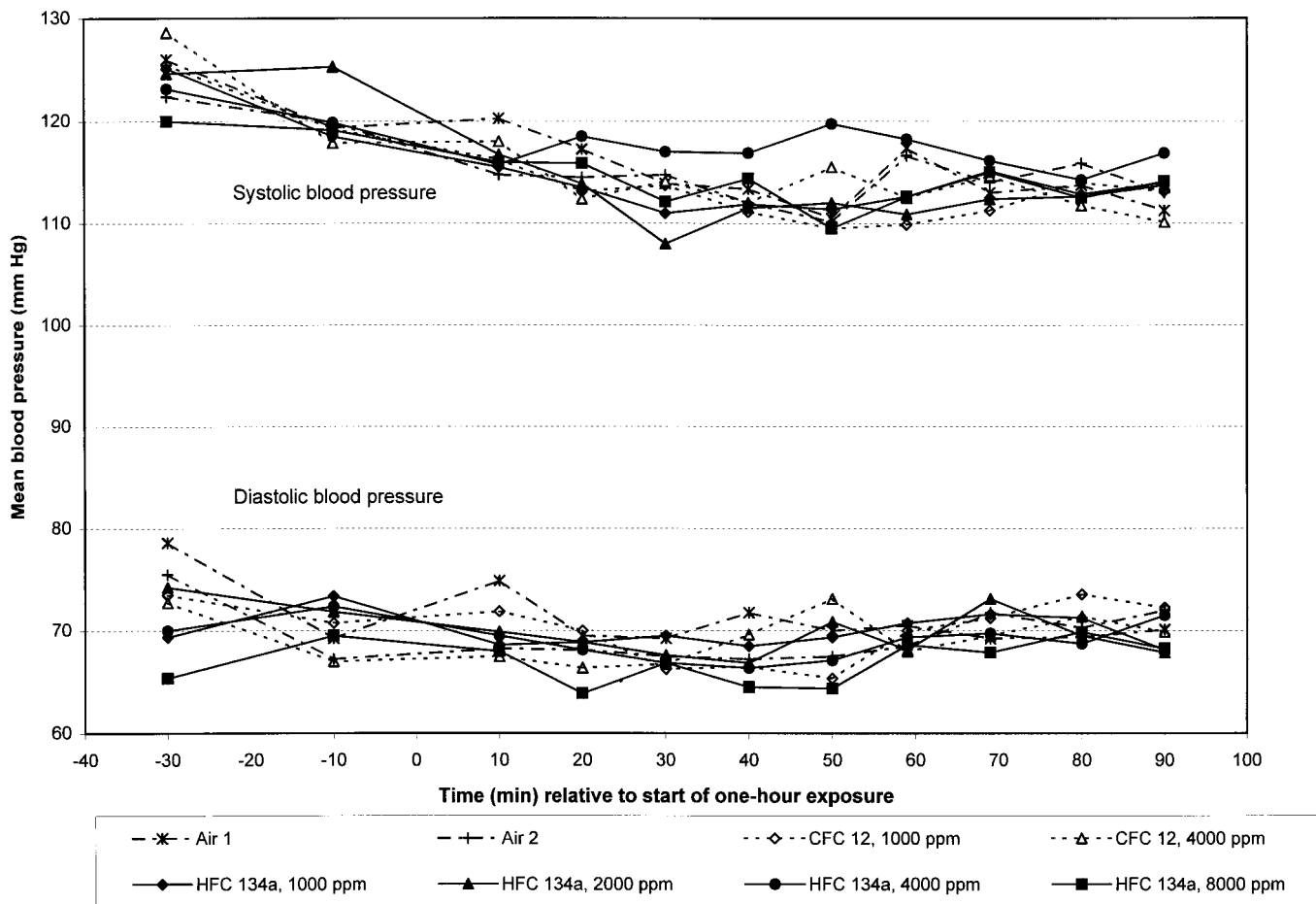


FIG. 1. Mean systolic and diastolic blood pressure ($n = 8$) measured prior to, during, and following 1-h whole-body exposure to HFC 134a (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

and a separate blood sample was taken for analysis of HFC 134a, HFC 227, or CFC 12.

• Blood samples taken for analysis of HFC 134a, HFC 227, or CFC 12 were collected via an indwelling cannula into heparinized syringes. Care was taken to avoid air bubbles. The entire contents of the syringe were transferred to sealed headspace vials (vial, Chromacol Type 6-CV; seal, Chromacol septa butylrubber type 20-B3P; caps, magnetic, gold, 20 mm; all three supplied by Alltech/Applied Science Group, Emmen, The Netherlands) which earlier had been weighed and had approximately 3.5 mL of air removed using a 10-mL syringe. Vials were weighed to determine sample weight. An internal standard, HFC 134 (1,1,2,2-tetrafluoroethane; 99% pure, Lancaster Synthesis Ltd, Morecombe, Great Britain; and ABCR GmbH & Co, Karlsruhe, Germany, both supplied by Brunshwig Chemie BV, Amsterdam, The Netherlands), 50 μ L (equivalent to 1.5 μ g), was added to each vial and the vials were then frozen (range -32 to -19°C) until analysis.

A separate blood sample was analysed for the following parameters:

• Hematology: White blood cell count, red blood cell count, hemoglobin, hematocrit, platelets, eosinophils, neutrophils, lymphocytes, monocytes, basophils, and other cells.

• Serum chemistry: Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ -glutamyltransferase, total bilirubin, creatinine, total protein, glucose, sodium, and potassium.

Urine samples were analyzed for white blood cell count, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, and blood.

Adverse events either observed or reported were recorded.

Whole blood samples were analyzed for HFC 134a, HFC 227, or CFC 12 using a Carlo Erba Model 4100 gas chromatograph equipped with a split/splitless injector, a flame ionization detector, and a headspace autosampler (Interscience BV, 4800 CC, Breda, The Netherlands). The headspace vials were placed in a

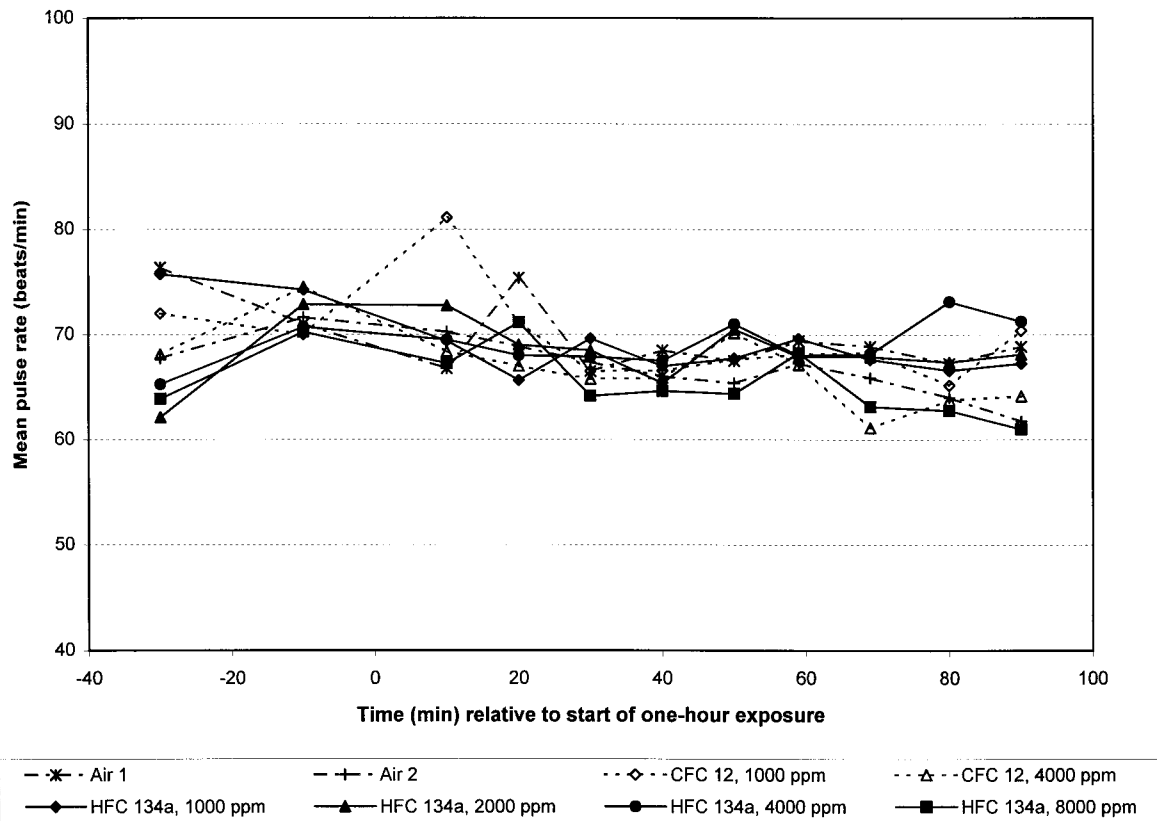


FIG. 2. Mean pulse rate ($n = 8$) measured prior to, during, and following 1-h whole-body exposure to HFC 134a (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

water bath at 60°C, with a minimum equilibration time of 30 min. One milliliter of gas in the headspace above the blood sample was injected with an autosampler Model HS 250 using splitless injection. A Poraplot Q column (Chrompack), 25 m \times 0.53 mm, with particles 10–20 μ m and a 2.5-m particle trap, was used. The column was operated initially at 50°C for 1 min, after which the temperature was increased at 20.0°C/min to 70°C; subsequently the temperature was increased at 5.0°C/min to 110°C (end of run). After each run, the temperature was increased at 30.0°C/min to 190°C and held for 5 min to clean the system. The injection port temperature was 200°C and the detector base temperature was 250°C. Splitless conditions were held for 30 s after injection and then changed to split mode. The carrier gas was helium with a column inlet pressure of 80 kPa. Data acquisition was performed using a Spectra Physics Chromnet/Labnet station.

Data Analysis

The primary safety variables were blood pressure (systolic and diastolic), pulse rate, lung function, and an evaluation of the electrocardiogram. Each HFC 134a or HFC 227 parameter was compared with CFC 12 and air for each equivalent time point, using paired Student's t

test (SAS Institute BV, Huizen, The Netherlands). Data from the two air exposures were pooled if there was no significant difference. If a significant difference was observed (P value < 0.05), the HFC 134a or HFC 227 comparison was made with the second air exposure (since by the time of the second air exposure, the volunteer would have become used to the chamber and the experimental procedures). Similarly, data from the two CFC 12 (1000 and 4000 ppm) exposures were pooled if there was no significant difference. If a significant difference was observed (P value < 0.05), the HFC 134a or HFC 227 comparison was made with the 4000 ppm CFC 12 exposure. The higher CFC 12 exposure concentration was selected since any possible effect would be expected to be of a greater magnitude and therefore any difference in the two propellants more readily determined. Further, for blood pressure and pulse rate, because a Student's t test was applied per time point (11 in total), a (Bonferroni) correction was made by dividing the 0.05 significance level by 11 giving a P value less than 0.0045 which was considered significant. Similarly, for lung function (PEF), after applying a correction for the two measurements, a P value less than 0.025 was considered significant.

Pharmacokinetic parameters C_{\max} (maximum blood

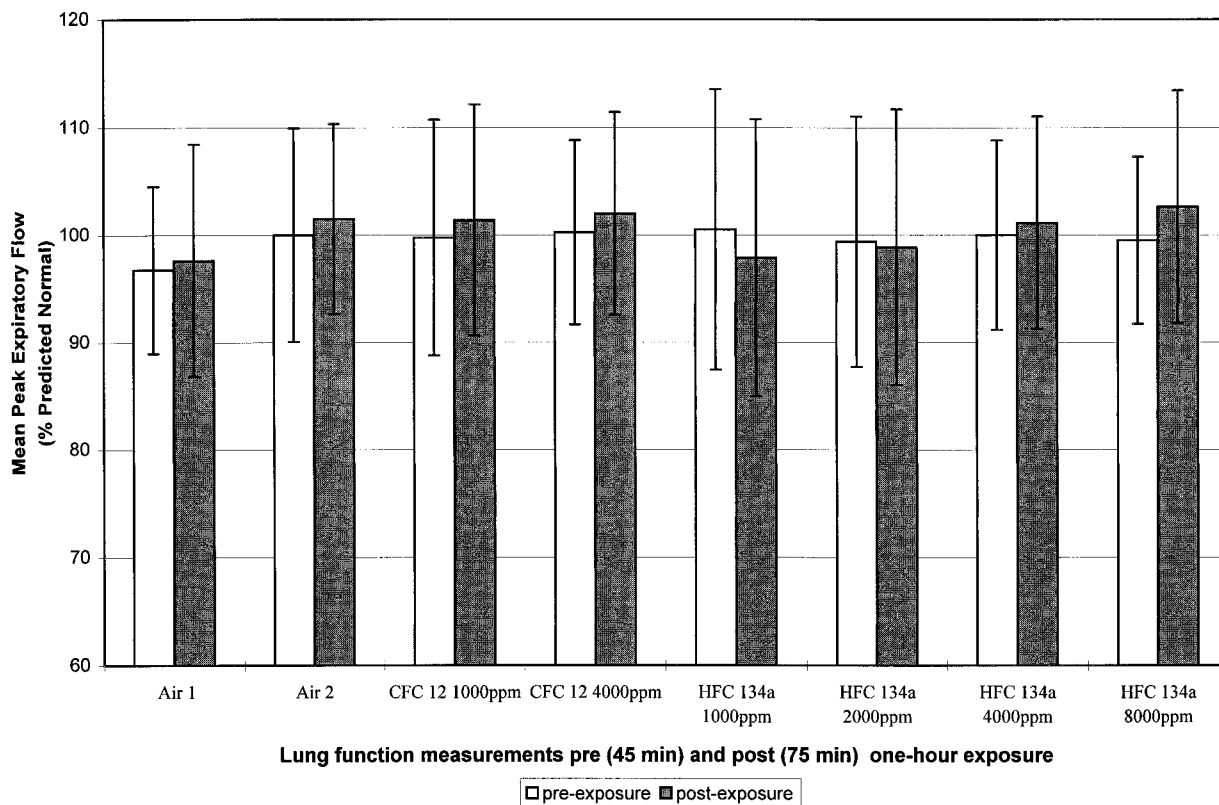


FIG. 3. Mean lung function ($n = 8$) measured prior to and following 1-h whole-body exposure to HFC 134a (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

concentration), T_{\max} (time to maximum blood concentration), $t_{1/2\alpha}$ (α elimination half-life), $t_{1/2\beta}$ (β elimination half-life), MRT (mean residence time from start of exposure to the last measured concentration time point), AUC_{0-t} (area under the concentration–time curve from start of exposure to the last measured concentration time point), and $AUC_{0-\infty}$ (from start of exposure to infinity) were calculated using TopFit (Version 2.0, Gustav Fischer Verlag, Stuttgart, Germany) by noncompartmental analysis. C_{\max} , T_{\max} , $t_{1/2\alpha}$, $t_{1/2\beta}$, MRT, and AUC_{0-t} were determined from the blood concentration–time profile and $AUC_{0-\infty}$ was estimated from AUC_{0-t} and extrapolation from the last measured time point. In a number of cases, only one elimination phase was apparent. This has been reported as $t_{1/2\beta}$, except for exposure to 1000 ppm HFC 227, where blood concentrations were rapidly below the limit of quantification and only $t_{1/2\alpha}$ is estimated. Male and female C_{\max} , AUC_{0-t} , and MRT values were compared at each exposure level using unpaired Student's t tests. A P value < 0.05 was considered significant.

RESULTS

HFC 134a

Measured exposure chamber concentrations were within 2% of the target exposure concentration and

stable (coefficient of variation less than 3%) throughout the 1-h exposure period. The overall ranges for chamber temperature and oxygen content were 22.6–24.0°C and 20.0–21.0%, respectively.

Systolic and diastolic blood pressure measurements (Fig. 1) showed no trend for a change as a result of exposure. For diastolic blood pressure at each time point, there were no statistically significant differences between exposure to HFC 134a (at any exposure level) compared to either air (combined) or CFC 12 (combined). On two occasions, systolic blood pressure was significantly different ($P < 0.0045$) between exposure to HFC 134a and air (combined). On one occasion it was lower compared to air (at 59 min during exposure to 1000 ppm HFC 134a) and on the other occasion, it was higher compared to air (at 50 min during exposure to 4000 ppm HFC 134a). Neither of these differences is considered clinically relevant since there was no trend with time nor exposure concentration. There were no other significant differences in systolic blood pressure.

As with the blood pressure measurement, pulse rate (Fig. 2) showed no trend or change with time as a result of exposure and at each time point there were no statistically significant differences between HFC 134a and air or CFC 12.

Peak expiratory flow, as a marker for lung function,

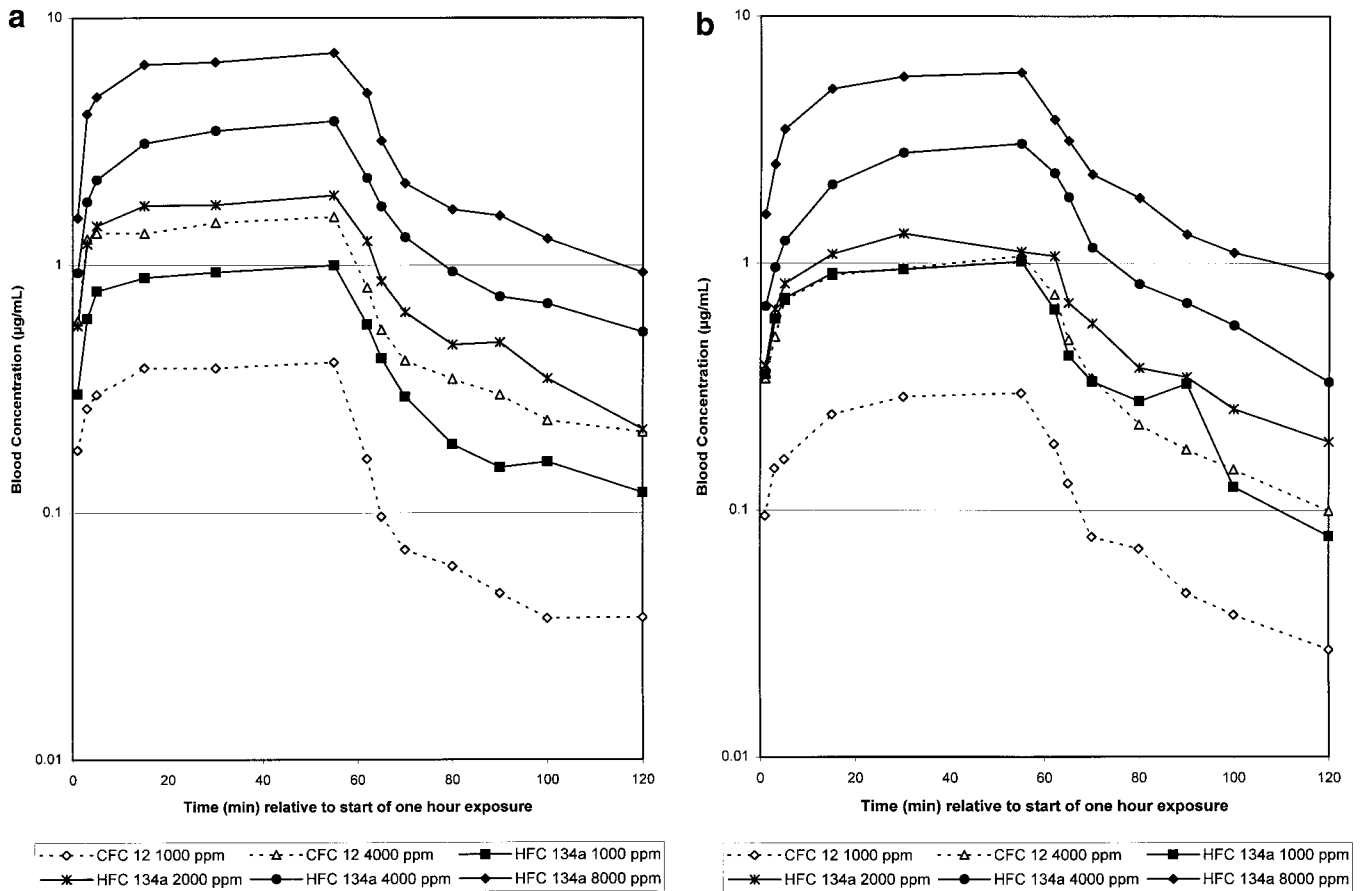


FIG. 4. (a) Mean blood concentrations in male volunteers ($n = 4$) during and following 1-h whole-body exposure to HFC 134a (1000, 2000, 4000, 8000 ppm) or CFC 12 (1000, 4000 ppm). (b) Mean blood concentrations in female volunteers ($n = 4$) during and following 1-h whole-body exposure to HFC 134a (1000, 2000, 4000, 8000 ppm) or CFC 12 (1000, 4000 ppm).

showed no difference between the pre- and postexposure values (Fig. 3). Additionally, for each time point there were no statistically significant differences between HFC 134a and air or CFC 12.

ECG was monitored by telemetry from between 15 and 45 min prior to exposure to 30 min after the end of exposure (60 min after the end of the final exposure). There were no clinically relevant changes in the ECG traces. The following are examples of non-treatment-related observations:

- Sinus arrhythmia was noted pre-, during, and/or postexposure in all subjects at some time (for individual subjects, not necessarily for each exposure condition) and for each exposure condition (although not each subject per exposure condition). Most of these occurred during either blood sampling and/or blood pressure measurement.
- Wenckebach phenomenon (Mobitz type I, second-degree heart block) in one subject during both exposure and nonexposure periods.
- Sinus bradycardia, in a few subjects during exposure to either test materials or air.

There were neither clinically significant changes in any of the laboratory parameters measured nor any notable adverse events recorded. Specifically there were no central nervous system effects reported or observed and there was no indication of upper respiratory tract irritation.

The blood concentration–time profiles of HFC 134a and CFC 12 (Fig. 4a, males, and Fig. 4b, females) show that both propellants were rapidly absorbed and cleared at each exposure concentration in both males and females. The mean (standard deviation) pharmacokinetic parameters for CFC 12 and HFC 134a in male and female volunteers are presented in Table 1.

Early in the study, a number of preexposure blood samples were found to contain quantifiable levels ($<0.10 \mu\text{g mL}^{-1}$) of either HFC 134a or CFC 12 (limits of quantitation 0.011 and $0.021 \mu\text{g mL}^{-1}$, respectively). While the source of this low-level contamination was never identified, all volunteer preexposure activities and assessments were performed in an area removed from the exposure chamber, to eliminate the possibility of low-level exposure.

TABLE 1
Mean ($n = 4$, SD) Male and Female Pharmacokinetic Parameters Following 1-h Whole-Body Exposure to Varying Concentrations of either HFC 134a or CFC 12

Exposure	Gender	C_{\max} ($\mu\text{g mL}^{-1}$)	T_{\max} (min)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	MRT (min)	AUC_{0-t} ($\mu\text{g} \cdot \text{min mL}^{-1}$)	$\text{AUC}_{0-\infty}$ ($\mu\text{g} \cdot \text{min mL}^{-1}$)
CFC 12								
1000 ppm	Female	0.33 (0.034)	30–55	6.6 ^a (1.6)	33.6 (10.2)	42.6 (1.07)	18.8 (2.2)	20.1 (2.0)
	Male	0.42 (0.088)	30–55	4.4 ^a (0.9)	47.5 (27.8)	38.8 (3.4)	25.3* (3.0)	27.6 (3.7)
4000 ppm	Female	1.1 (0.20)	30–55	9.6 (3.5)	36.6 (10.7)	43.1 (4.3)	68.8 (9.9)	73.9 (9.2)
	Male	1.6* (0.14)	30–55	6.7 (1.7)	25.4 (11.6)	41.5 (3.3)	104.7* (5.8)	112.3 (7.9)
HFC 134a								
1000 ppm	Female	1.0 (0.17)	55	5.7 ^a (0.76)	39.1 (19.7)	43.1 (3.6)	69.9 (10.3)	74.1 (7.9)
	Male	1.0 (0.18)	30–55	9.8 (4.3)	37.8 (12.7)	41.8 (4.6)	66.3 (9.7)	73.6 (10.8)
2000 ppm	Female	1.4 (0.40)	30–55	9.7 ^b	57.6 (63.6)	45.3 (4.2)	90.3 (20.8)	110.5 (47.6)
	Male	1.9 (0.40)	55	9.0 ^b	34.3 (12.7)	43.7 (4.0)	131.8 (27.0)	142.0 (28.0)
4000 ppm	Female	3.1 (0.18)	30–55	11.1 (3.9)	31.8 (10.1)	46.2 (2.5)	193.4 (5.5)	209.4 (9.3)
	Male	3.8 (0.47)	55	11.2 (4.2)	57.8 (34.7)	44.4 (6.4)	247.3* (24.1)	304.9 (81.3)
8000 ppm	Female	6.0 (0.53)	30–55	9.4 ^b	44.5 (34.1)	45.0 (1.8)	405.8 (16.5)	462.2 (42.7)
	Male	7.2* (0.70)	55	8.3 (1.2)	38.3 (2.3)	43.4 (1.7)	482.5 (68.0)	534.2 (82.4)

Note. C_{\max} , maximum blood concentration; T_{\max} , time to maximum blood concentration; $t_{1/2\alpha}$, α elimination half-life; $t_{1/2\beta}$, β elimination half-life; MRT, mean residence time from start to the last measured concentration time point; AUC_{0-t} , area under the total blood concentration–time curve; $\text{AUC}_{0-\infty}$, total area under the blood concentration–time curve extrapolated from last measured blood concentration.

^a $n = 3$, phase not apparent in one volunteer.

^b $n = 2$, phase not apparent in two volunteers.

* Significant difference between males and females, $P < 0.05$.

For both HFC 134a and CFC 12, in males and females, blood concentrations increased rapidly and at 15 min were near maximum concentrations at each exposure concentration. Blood concentrations approached steady state, asymptotically, with maximum blood concentrations measured generally (>80%) in the final sample taken during exposure. Both maximum concentrations and total AUC increased in relation to exposure concentration (Table 1), although the increases were not strictly proportional. There was a tendency for a gender difference, with both these parameters being lower in females. For CFC 12, maximum blood concentrations were 27 and 44% higher in males for 1000 and 4000 ppm exposure levels, respectively. The difference was statistically significant ($P < 0.05$) at the 4000 ppm exposure level only. The gender difference in CFC 12 AUC was statistically significant ($P < 0.05$) at both exposure levels, with values being 34 (1000 ppm) and 52% (4000 ppm) higher in males. Maximum blood concentrations and AUC for HFC 134a, 1000 ppm, were similar in males and females. Maximum blood concentrations were 33, 23, and 21% higher in males for 2000, 4000, and 8000 ppm HFC 134a exposure levels, respectively. The difference was statistically significant only at 4000 and 8000 ppm ($P < 0.05$). HFC 134a AUC values were 45, 27, and 22% higher in males, for 2000, 4000, and 8000 ppm exposure levels, respectively, although the difference was statistically significant ($P < 0.05$) at only 4000 ppm. Total AUC and AUC to the last measured blood sample indicate that the profiles were adequately followed

since, on average, only 10% of the total curve was estimated by extrapolation.

Elimination of HFC 134a and CFC 12 was predominantly (>75%) biphasic at all exposure levels and independent of both gender and exposure concentration. In the remainder of the population, a single elimination phase was observed. The CFC 12 $t_{1/2\alpha}$ was a mean of 7 min, and $t_{1/2\beta}$ a mean of 36 min. The HFC 134a $t_{1/2\alpha}$ was also short, a mean of 9 min, and $t_{1/2\beta}$ was a mean of 42 min. As with half-lives, MRT was independent of gender and exposure concentration. MRT was a mean of 42 and 44 min for CFC 12 and HFC 134a, respectively.

Following the final exposure a 24-h blood sample was analyzed for HFC 134a or CFC 12. The sample of one volunteer, whose final exposure had been to 8000 ppm HFC 134a, had blood concentrations of $0.364 \mu\text{g mL}^{-1}$. The remaining samples were below the limit of quantification (HFC 134a, $0.011 \mu\text{g mL}^{-1}$, and CFC 12, $0.021 \mu\text{g mL}^{-1}$).

HFC 227

Measured exposure chamber concentrations were within 2% of the target exposure concentration and stable (coefficient of variation less than 4%) throughout the 1-h exposure period. The overall ranges for chamber temperature and oxygen content were 22.4–23.6°C and 20.4–20.9%, respectively.

Systolic and diastolic blood pressure measurements (Fig. 5) showed no trend for a change as a result of exposure. At each time point, there were no statisti-

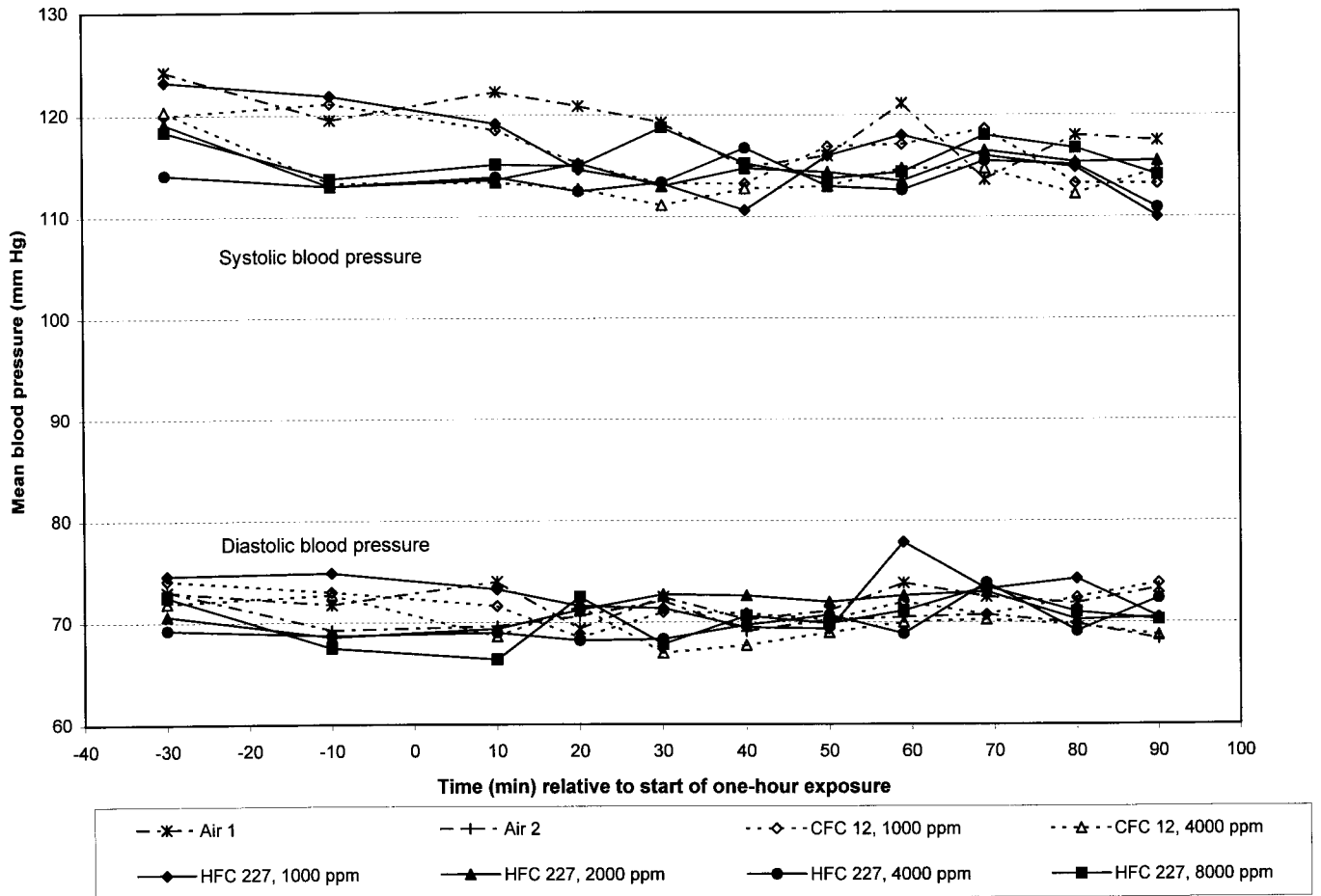


FIG. 5. Mean systolic and diastolic blood pressure ($n = 8$) measured prior to, during, and following 1-h whole-body exposure to HFC 227 (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

cally significant differences between exposure to HFC 227 (at any exposure level) compared to air (combined) or CFC 12 (combined).

As with the blood pressure measurement, pulse rate (Fig. 6) showed no trend or change with time as a result of exposure and at each time point there were no statistically significant differences between HFC 227 and air or CFC 12.

Peak expiratory flow, as a marker for lung function, showed no difference between the pre- and postexposure values (Fig. 7). Additionally, for each time point there were no statistically significant differences between HFC 227 and air or CFC 12.

ECG was monitored by telemetry from between 15 and 45 min prior to exposure to 30 min after the end of exposure (60 min after the end of the final exposure).

There were no clinically relevant changes in the ECG traces. The following are examples of non-treatment-related observations:

- Sinus arrhythmia was noted pre- during, and/or postexposure in all subjects at some time (for individual subjects, not necessarily for each exposure condi-

tion) and for each exposure condition (although not each subject per exposure condition). Most of these occurred during either blood sampling and/or blood pressure measurement.

- Sinus bradycardia, in a few subjects during exposure to either test materials or air.
- Sinus tachycardia, before, during, and/or after exposure to either test materials or air.

There were neither clinically significant changes in any of the laboratory parameters measured nor any notable adverse events recorded. Specifically there were no central nervous system effects reported or observed and there was no indication of upper respiratory tract irritation.

The blood concentration-time profiles of HFC 227 and CFC 12 (Fig. 8a, males, and Fig. 8b, females) show that both propellants were rapidly absorbed and cleared at each exposure concentration in both males and females. Mean pharmacokinetic parameters for CFC 12 and HFC 227 in male and female volunteers are presented in Table 2.

Early in the study, a number of preexposure blood

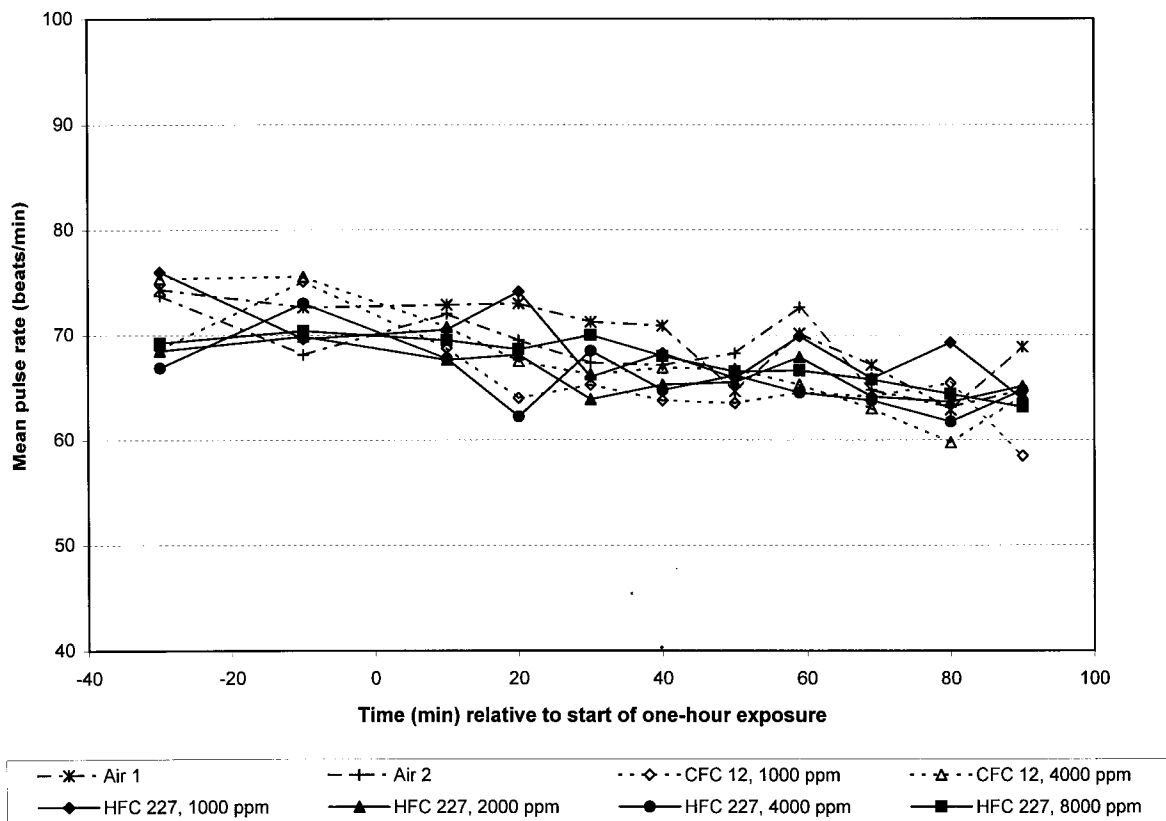


FIG. 6. Mean pulse rate ($n = 8$) measured prior to, during, and following 1-h whole-body exposure to HFC 227 (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

samples were found to contain quantifiable levels ($<0.10 \mu\text{g mL}^{-1}$) of either HFC 227 or CFC 12 (limits of quantitation 0.010 and $0.021 \mu\text{g mL}^{-1}$, respectively). While the source of this low-level contamination was never identified, volunteers had preexposure activities and assessments performed in an area away from the exposure chamber.

Blood concentrations of both HFC 227 and CFC 12, in males and females, increased rapidly and at 15 min were either maximum (13% of the cases) or near maximum at each exposure concentration. Blood concentrations appeared to be at steady state, with maximum blood concentrations generally measured (52% of the cases) prior to the final sample taken during exposure. Both maximum concentrations and total AUC increased in relation to exposure concentration (Table 2), although the increases were not strictly proportional. At each exposure level, and for both CFC 12 and HFC 227, maximum blood concentrations were statistically significantly higher in males ($P < 0.05$) than females. For CFC 12 maximum blood concentrations were 54 and 63% higher in males at 1000 and 4000 ppm, respectively. For HFC 227, maximum blood concentrations were 37, 50, 39, and 31% higher in males at 1000, 2000, 4000, and 8000 ppm, respectively. In accordance with blood concentration, AUC was statistically signifi-

cantly higher in males ($P < 0.05$) than females, except at 1000 ppm HFC 227. The CFC 12 AUC was 54 and 58% higher in males at 1000 and 4000 ppm, respectively, and HFC 227 AUC was 30, 52, 34, and 41% higher in males at 1000, 2000, 4000, and 8000 ppm, respectively. Total AUC and AUC to the last measured blood sample indicate that the profiles were adequately followed since, on average, less than 10% of the total curve was estimated by extrapolation.

Elimination of HFC 227 and CFC 12 was predominantly ($>83\%$) biphasic at each exposure level, gender dependent, and exposure concentration independent. In the remainder of the population, a single elimination phase was observed. Elimination appeared to be independent of exposure levels. For both CFC 12 and HFC 227, at each exposure level, $t_{1/2\alpha}$ was short and tended to be lower in males than females. For CFC 12 $t_{1/2\alpha}$ was a mean (of both exposure levels) of 6.3 and 9.2 min for males and females, respectively. For HFC 227 $t_{1/2\alpha}$ was a mean (of all exposure levels) of 4.7 and 7.9 min for males and females, respectively. For CFC 12 $t_{1/2\beta}$ was similar, approximately 40 min, for males and females at 1000 ppm and males at 4000 ppm exposure concentrations. For females following exposure to 4000 ppm CFC 12, $t_{1/2\beta}$ was 23 min. For HFC 227 $t_{1/2\beta}$ was variable in both males and females. For females, fol-

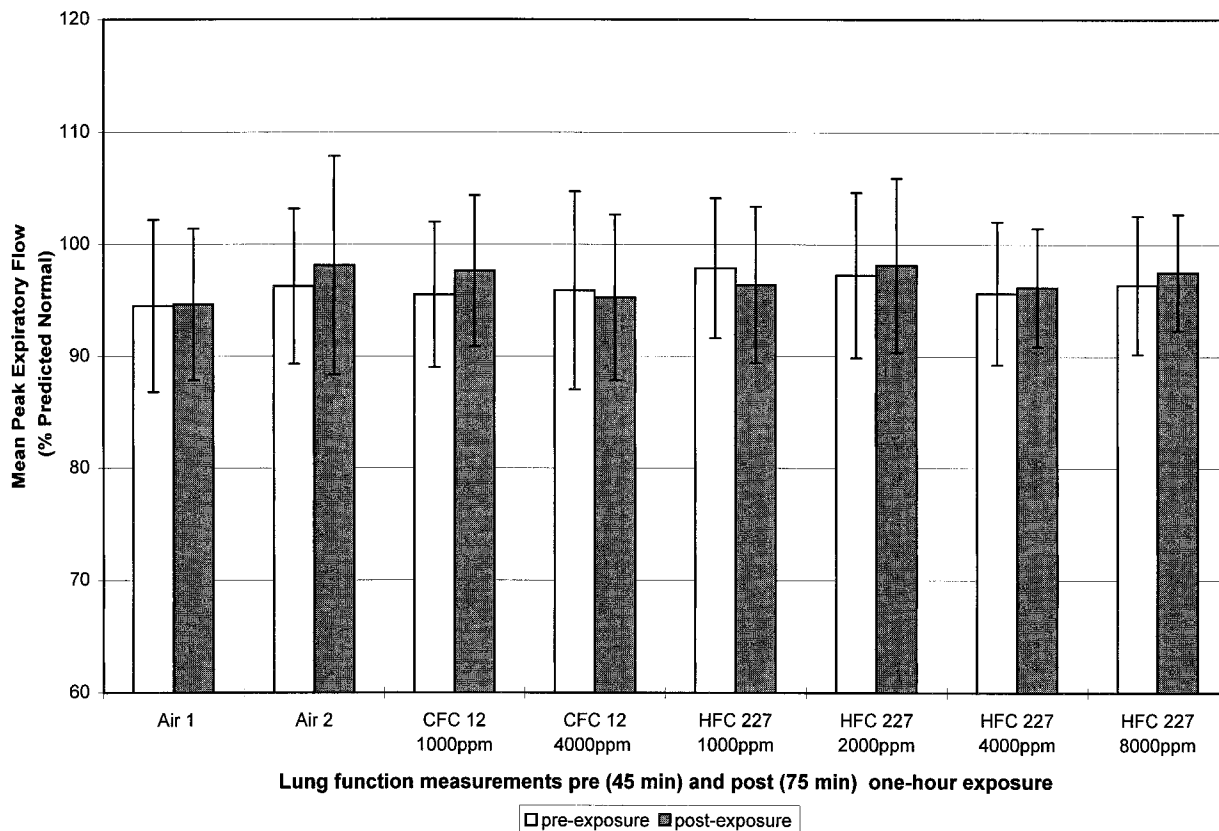


FIG. 7. Mean lung function ($n = 8$) measured prior to and following 1-h whole-body exposure to HFC 227 (1000, 2000, 4000, 8000 ppm), CFC 12 (1000, 4000 ppm), or air.

lowing exposure to 1000 and 8000 ppm HFC 227, $t_{1/2}\beta$ was approximately 22 min and following exposure to 2000 and 4000 ppm, it was approximately 42 min. For males, $t_{1/2}\beta$ was similar, approximately 37 min, following exposure to 1000 and 8000 ppm HFC 227, and it was 62 and 92 min following exposure to 2000 and 4000 ppm HFC 227, respectively.

For both HFC 227 and CFC 12, MRT was statistically significantly ($P < 0.05$) lower in males than females at each exposure level and independent of exposure concentration. For CFC 12, MRT was a mean of 37 and 45 min for males and females, respectively. For HFC 227, MRT was a mean of 36 and 42 min for males and females, respectively.

Following the final exposure a 24-h blood sample was analyzed for HFC 227 or CFC 12. All samples were below the limit of quantification (HFC 227, $0.010 \mu\text{g mL}^{-1}$, and CFC 12, $0.021 \mu\text{g mL}^{-1}$).

CONCLUSIONS

HFC 134a and HFC 227 have been separately evaluated in a series of toxicological and clinical studies. The animal studies have demonstrated that HFC 134a exhibits only minimal toxic properties even with

2-year, 5 days per week, 6-h exposure to levels of 50,000 ppm (Collins *et al.*, 1995; Alexander *et al.*, 1995; ECETOC, 1995) and that HFC 227 exhibits only minimal toxic properties even with 2-year daily 1-h exposure to levels of 240,000 ppm (IPACT-II, unpublished studies; Skaggs *et al.*, 1995). The clinical trials, which have evaluated HFC 134a and HFC 227 as replacement propellants for CFC 12 in MDIs, again have demonstrated the safety of these products. In fact, no adverse effects were seen in volunteers who received multiple exposures to HFC 134a and HFC 227 at doses much higher than would be delivered therapeutically (Alexander and Libretto, 1995; Alexander, 1995; IPACT-II, unpublished studies).

The report of Vinegar *et al.* (1997)—suggesting that short-term exposure of a healthy volunteer to a level of 4000 ppm HFC 134a could cause loss of consciousness and that short-term exposure of a healthy volunteer to a level of 6400 ppm HFC 227 could cause a rapid increase in pulse rate—ran counter to all previous findings. The present studies were therefore designed to monitor the effects associated with exposure to HFC 134a or HFC 227 at higher levels and for longer periods of time than were used in the study reported by Vinegar *et al.* In the current studies, each subject was

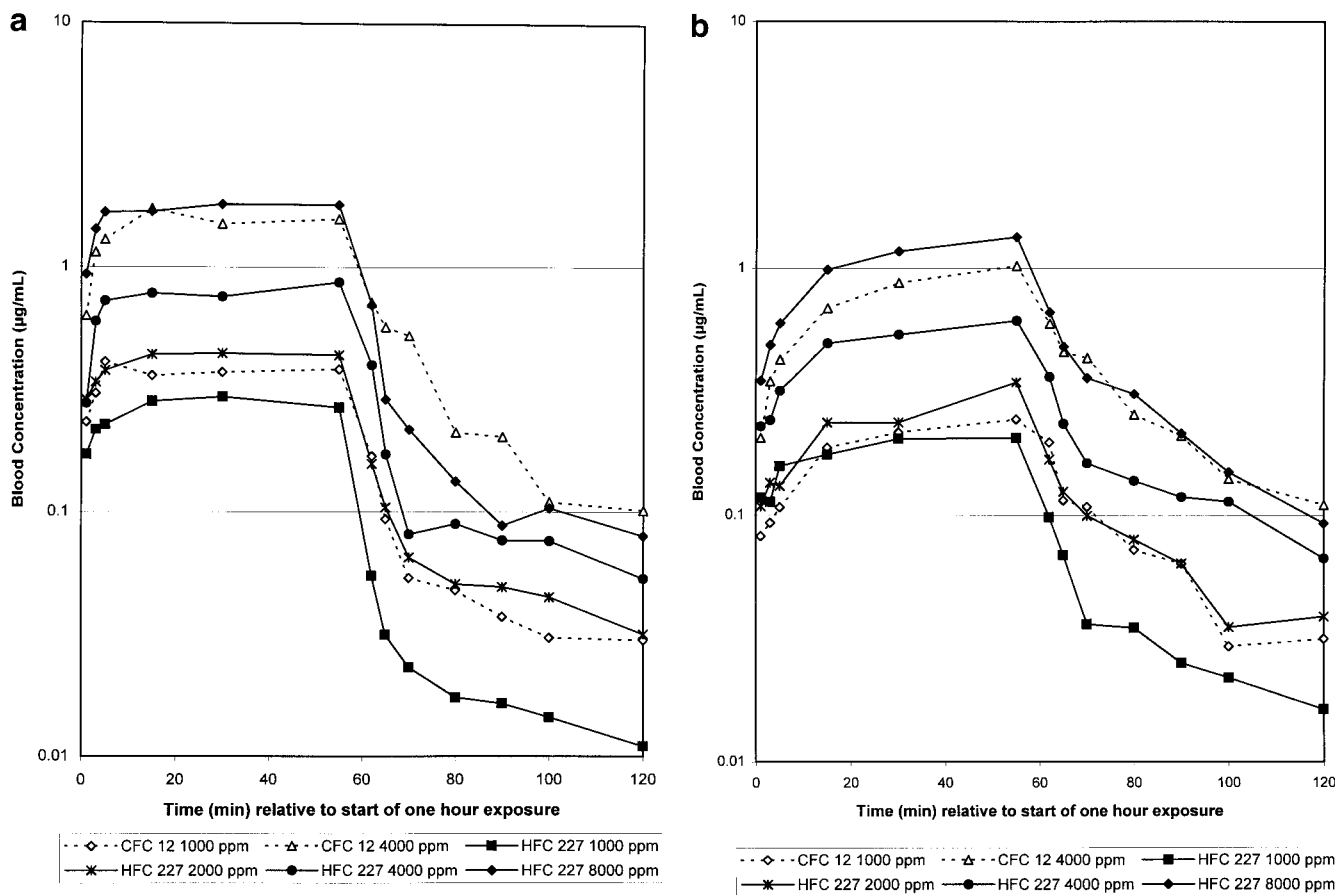


FIG. 8. (a) Mean blood concentrations in male volunteers ($n = 4$) during and following 1-h whole-body exposure to HFC 227 (1000, 2000, 4000, 8000 ppm) or CFC 12 (1000, 4000 ppm). (b) Mean blood concentrations in female volunteers ($n = 4$) during and following 1-h whole-body exposure to HFC 227 (1000, 2000, 4000, 8000 ppm) or CFC 12 (1000, 4000 ppm).

exposed whole body, first to air and then to ascending HFC 134a or HFC 227 concentrations of 1000, 2000, 4000, and 8000 ppm each for 1 h. Interspersed among these exposures were a second exposure to air and two exposures to CFC 12 (at 1000 and 4000 ppm). Lung function (PEF) was measured prior to and following each exposure. Pulse, ECG, and blood pressure were monitored and blood samples were collected prior to, during, and following each exposure. Observed or reported adverse events were recorded. Blood concentrations of HFC 134a, HFC 227, and CFC 12 were measured, and the uptake and elimination curves were determined.

CFC 12 was chosen as the control because of its history of use as a propellant in past and current MDIs. This allowed for direct comparisons of HFC 134a to CFC 12 and of HFC 227 to CFC 12. As can be seen from the data, exposure to CFC 12, HFC 134a, and HFC 227 had no effect on blood pressure (Figs. 1 and 5) or pulse (Figs. 2 and 6). ECG recordings did not show any effects that appeared related to CFC 12, HFC 134a, or HFC 227 exposures. All subjects displayed pre-, during, and postexposure events of sinus arrhythmia.

Most of these observations were parallel with blood sampling or blood pressure measurements. Additionally, reactions were seen occasionally during insertion of the canulla, especially during the first or second exposure. No difficulties were experienced in the collection of the blood samples. Lung function did not show any evidence of treatment-related effects (Figs. 3 and 7).

The pattern of uptake for HFC 134a, HFC 227, and CFC 12 was similar for males and females (Figs. 4a, 4b, 8a, and 8b). However, actual blood concentrations of each compound tended to be higher in males compared to females. This may be due to the difference in body fat between males and females. In females, the body fat was higher than in males and lower female blood concentrations may be a result of greater distribution into body fat. Elimination was generally rapid and biphasic for HFC 134a, HFC 227, and CFC 12 and independent of gender in the HFC 134a study but gender dependent in the HFC 227 study. The rapid elimination is typical of poorly soluble materials with high vapor pressures and demonstrated the lack of potential to bioaccumulate. This has previously been reported for HFC 134a

TABLE 2
Mean ($n = 4$, SD) Male and Female Pharmacokinetic Parameters Following 1-h Whole-Body Exposure to Varying Concentrations of either HFC 227 or CFC 12

Exposure	Gender	C_{\max} ($\mu\text{g mL}^{-1}$)	T_{\max} (min)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	MRT (min)	AUC_{0-t} ($\mu\text{g} \cdot \text{min mL}^{-1}$)	$\text{AUC}_{0-\infty}$ ($\mu\text{g} \cdot \text{min mL}^{-1}$)
CFC 12								
1000 ppm	Female	0.26 (0.047)	30–55	9.4 ^a	40.8 (49.0)	45.3 (2.7)	15.8 (2.9)	17.6 (2.0)
	Male	0.40* (0.066)	15–55	5.3 ^b (0.38)	39.9 (32.0)	36.5* (1.7)	24.2* (2.7)	25.7 (1.5)
4000 ppm	Female	1.1 (0.24)	30–55	9.1 ^a	23.3 (9.0)	45.6 (1.5)	62.1 (10.2)	65.8 (11.1)
	Male	1.7* (0.22)	15–55	7.3 (4.5)	42.5 (16.1)	38.2* (3.3)	97.9* (14.6)	104.0 (12.6)
HFC 227								
1000 ppm	Female	0.27 (0.020)	30–55	6.5 (2.3)	19.1 ^b (3.9)	37.4 (1.9)	12.9 (1.6)	13.0 (1.8)
	Male	0.31* (0.059)	15–55	4.4 (2.3)	39.1 ^b (22.9)	33.1* (2.7)	16.8 (3.9)	17.1 (3.4)
2000 ppm	Female	0.35 (0.076)	55	8.9 (2.1)	43.0 (11.2)	45.4 (3.7)	18.9 (4.5)	21.5 (5.7)
	Male	0.52* (0.069)	30–55	5.8 (2.4)	62.2 (27.3)	36.9* (0.98)	28.7* (2.7)	31.5 (3.8)
4000 ppm	Female	0.63 (0.15)	15–55	8.1 (1.9)	40.6 (16.5)	44.3 (0.85)	38.5 (6.7)	42.5 (4.7)
	Male	0.88* (0.106)	30–55	4.5 (0.43)	92.2 (80.7)	37.5* (2.3)	51.5 (8.1)	57.9 (9.8)
8000 ppm	Female	1.4 (0.17)	15–55	7.9 ^b (0.54)	24.9 (5.4)	42.8 (2.8)	78.9 (6.5)	82.2 (6.1)
	Male	1.9* (0.09)	30–55	4.0 (1.6)	35.5 (11.0)	35.0* (1.6)	111.3* (9.1)	115.3 (10.2)

Note. C_{\max} , maximum blood concentration; T_{\max} , time to maximum blood concentration; $t_{1/2\alpha}$, α elimination half-life; $t_{1/2\beta}$, β elimination half-life; MRT, mean residence time from start to the last measured concentration time point; AUC_{0-t} , area under the total blood concentration–time curve; $\text{AUC}_{0-\infty}$, total area under the blood concentration–time curve, extrapolated from last measured blood concentration.

^a $n = 2$, phase not apparent in two volunteers.

^b $n = 3$, phase not apparent in one volunteer.

* Significant difference between males and females, $P < 0.05$.

in metabolism studies conducted with rats (Ellis *et al.*, 1993) and humans (Pike *et al.*, 1995). The biphasic elimination pattern and half-life of approximately 30 min have been reported previously (Pike *et al.*, 1995) in a study using ¹⁸F-labeled HFC 134a. They also reported that HFC 134a was distributed throughout the body in a uniform pattern. The rate of uptake, distribution, and elimination was similar for healthy subjects and patients with chronic airflow limitations. This current work with HFC 134a confirms much of these early reports; it also allows for estimation of blood levels over a 1-h time period. While our results tended to be more uniform than those reported by Pike *et al.* (1995), subjects in this study were younger and may have been more homogeneous. Also, continuous 1-h exposure design would allow for more uniform uptake compared to the single breath administration used in the Pike *et al.* study. Further support for the rapid clearance was seen in the 24-h post-final-exposure blood concentrations. In all cases except one, blood concentrations were below the limits of detection. Even in the one remaining sample, the blood concentration was 0.364 $\mu\text{g mL}^{-1}$ or approximately 3% of the peak level seen during the 8000 ppm HFC 134a exposure. It is unlikely that HFC 134a would accumulate in the body following multiple exposures since it is rapidly cleared primarily by exhalation and undergoes minimal metabolism (Ventresca, 1995).

While blood concentrations clearly followed an exposure-related pattern, it was not linear for either males or females. At the higher exposure levels, blood con-

centrations were lower than would have been predicted based on blood concentrations at the lower exposure levels. This may be related to the low solubility and high vapor pressures of HFC 134a and HFC 227. This observation is important for predicting blood concentrations associated with short, high-level exposures since simple linear extrapolations will overestimate blood concentrations.

In conclusion, exposure of healthy volunteers to exposure levels up to 8000 ppm HFC 134a and HFC 227 and up to 4000 ppm CFC 12 did not result in any adverse effects on pulse, blood pressure, ECG, or lung function. The comparability of the effects seen with these three compounds supports the conclusion that HFC 134a and HFC 227 are suitable replacements for CFC 12. The absence of adverse effects in this study further supports the conclusion that the findings reported by Vinegar *et al.* (1997) represent a spurious event unrelated to the inhalation of either HFC 134a or HFC 227.

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