Determination of Fluorinated Hydrocarbon Propellants in Blood of Dogs after Aerosol Administration

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Abstract ☐ A GC method utilizing electron-capture detection is described for the measurement of certain fluorinated chloromethane and chloroethane propellants in dog blood. The lower limits of quantitation were 3.3, 10, 40, and 80 ng./ml. of blood for trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane, respectively. These fluorocarbons were determined simultaneously in the arterial and venous blood of anesthetized male and female dogs following administration (by inhalation) of an aerosol mixture containing 25% (w/w) of each propellant. Trichloromonofluoromethane demonstrated the highest blood levels and declined slowly, whereas dichlorodifluoromethane and dichlorotetrafluoroethane showed lower blood concentrations and declined more rapidly. Trichlorotrifluoroethane declined at a rate that was intermediate between trichloromonofluoromethane and dichlorodifluoromethane.

Keyphrases ☐ Propellants, fluorinated hydrocarbon—analysis in blood after inhalation, dogs ☐ Fluorinated hydrocarbon propellants—analysis in blood after inhalation, dogs ☐ Blood, fluorinated hydrocarbon content—analysis after propellant inhalation, dogs ☐ GC, electron-capture detector—analysis, fluorinated hydrocarbons, blood, dogs

There has been increasing concern as to the possible toxicity of the fluorinated hydrocarbons used as propellants in bronchodilator (1–3) and other aerosols (4). Studies relating to the potential toxicity of these fluorocarbons require the availability of sensitive assays for the determination of blood levels. Hence, analytical methodology was developed and applied to the measurement of these fluorocarbons in the blood of mongrel dogs treated with intratracheal doses of propellants from an aerosol preparation.

EXPERIMENTAL

Materials—All fluorocarbons including trichloromonofluoromethane (b.p. 23.7°), dichlorodifluoromethane (b.p. −29.8°), 1,2-dichlorotetrafluoroethane (b.p. 4.1°), and trichlorotrifluoroethane (b.p. 47°) were purchased from a commercial source[1]. A pressurized aerosol was prepared containing 25% (w/w) of each fluorocarbon. The aerosol (15-ml. container) was fitted with a 50-μl. metered valve[2]. Each actuation of the valve delivered 67.2 mg. of fluorocarbons equal to 16.8 mg. each of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane.

High purity normal hexane[3] and vacutainers, 5-ml. draw containing 12.5 mg. potassium oxalate, were used[4].

Dosing of Animals and Collection of Blood Samples—Adult, nonfasted mongrel dogs of both sexes, weighing 8.8–14.2 kg., were anesthetized with sodium pentobarbital[5], 30 mg./kg. i.v. The trachea was exposed, cannulated, and fitted with a plastic adapter to fit the aerosol preparation. Blood samples were taken from both the right femoral vein and the right femoral artery *via* implanted polyethylene catheters. Animals were dosed by pressing the aerosol container (attached to a plastic adapter) to activate the metered valve. Ten successive actuations were delivered to each dog, one actuation on each successive inspiratory phase of respiration. Blood samples were removed using heparinized disposable 1.0-ml. syringes at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 10, 15, 30, 60, 90, and 120 min. after the first actuation. The heparinized blood sample (1.0 ml.) was injected into an ice-cold, 5-ml. draw vacutainer containing 12.5 mg. potassium oxalate and 2 or 4 ml. of hexane. The vacutainer was mixed thoroughly and placed on dry ice until analyzed.

GC Analysis—A dual-column gas chromatograph[6] equipped with a tritium foil electron-capture detector was used for all analyses. The fluorocarbons were chromatographed on a 1.83-m. (6-ft.) presilanized[7] glass column (3-mm. i.d.) packed with Poropak Q[8]. The column temperature was 140°, the flash heater temperature was 165°, and the temperature of the electron-capture detector was 190°. The purge gas was a mixture of 10% methane in argon, with a flow rate of 100 ml./min. The flow rate of the carrier gas (helium) was 25 ml./min. The pulse was set at 50 μsec.

Samples (3-μl. size) were injected in the gas chromatograph using a 10-μl. syringe[9]. Sample injections were performed as follows: initially, 1 μl. of solvent hexane was drawn into the syringe, followed by 1 μl. of air; then 3 μl. of the hexane extract was drawn, all into the syringe barrel, leaving air in the needle. Once retention times were established for individual standards, the fluorocarbons were pooled during the dilutions. The retention times were: dichlorodifluoromethane, 1.9 min.; dichlorotetrafluoroethane, 3.8 min.; trichloromonofluoromethane, 7.2 min.; and trichlorotrifluoroethane, 14 min. Hexane alone gave two peaks, with retention times of 16.5 and 15 min. (Fig. 1).

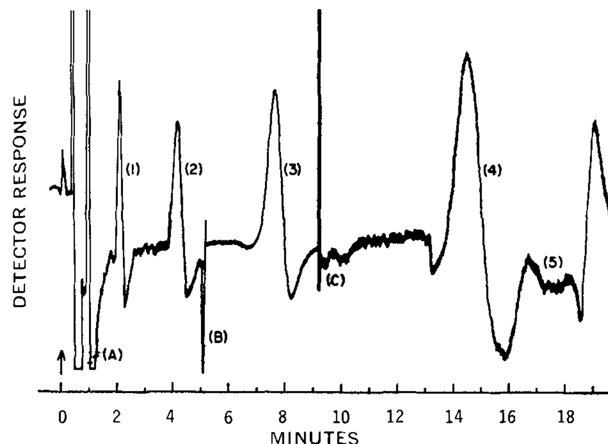

Figure 1—*Typical gas chromatogram of standards. Key: (1) dichlorodifluoromethane, 0.48 ng.; (2) dichlorotetrafluoroethane, 3.2 ng.; (3) trichloromonofluoromethane, 0.48 ng.; (4) trichlorotrifluoroethane, 3.2 ng.; and (5) hexane, 3 μl. Attenuations were: (A) 1 × 16, (B) 1 × 64, and (C) 1 × 16. See* Experimental *for chromatographic conditions.*

[1] Purchased as Freons from E. I. duPont de Nemours & Co., Wilmington, Del.
[2] Rikers Labs, Northridge, Calif.
[3] Phillips Petroleum Co., Bartlesville, Okla.
[4] Becton, Dickinson & Co., Rutherford, N. J.
[5] Nembutal, Abbott Labs.

[6] F & M model 400.
[7] The procedure for silanization was as follows. Fill the column with a 5% solution of dichlorodimethylsilane in reagent grade toluene. Allow to stand for 20 min., drain, rinse with reagent grade methanol, and allow to dry.
[8] Waters Associates, Framingham, Mass.
[9] Hamilton Co., Whittier, Calif.

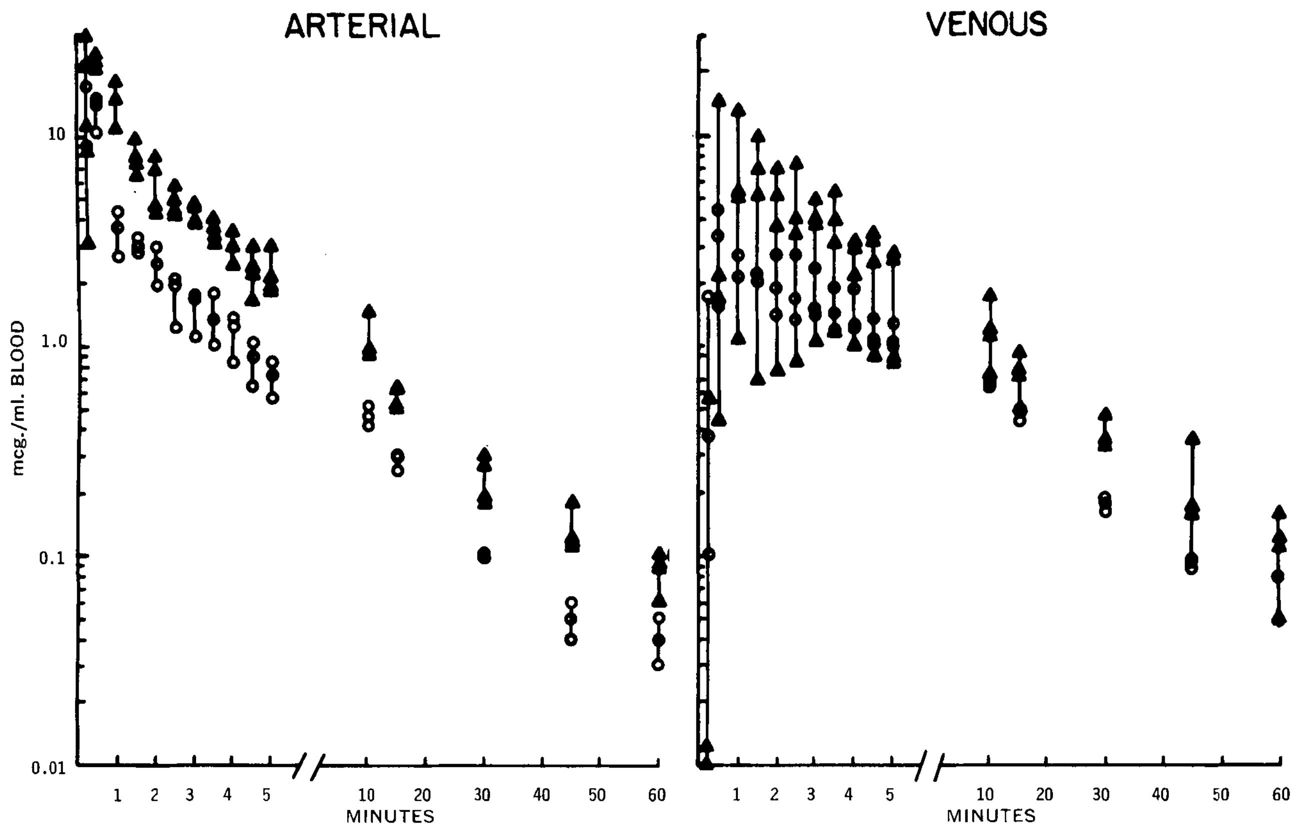

Figure 2—*Trichloromonofluoromethane blood levels in the dog. Five or 10 actuations of an aerosol mixture containing 25% (w/w) of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were administered intratracheally to three or four dogs, one actuation on each successive inspiratory phase of respiration. Each point represents the fluorocarbon blood level obtained from a single dog. Key:* ○, *five actuations; and* ▲, *10 actuations.*

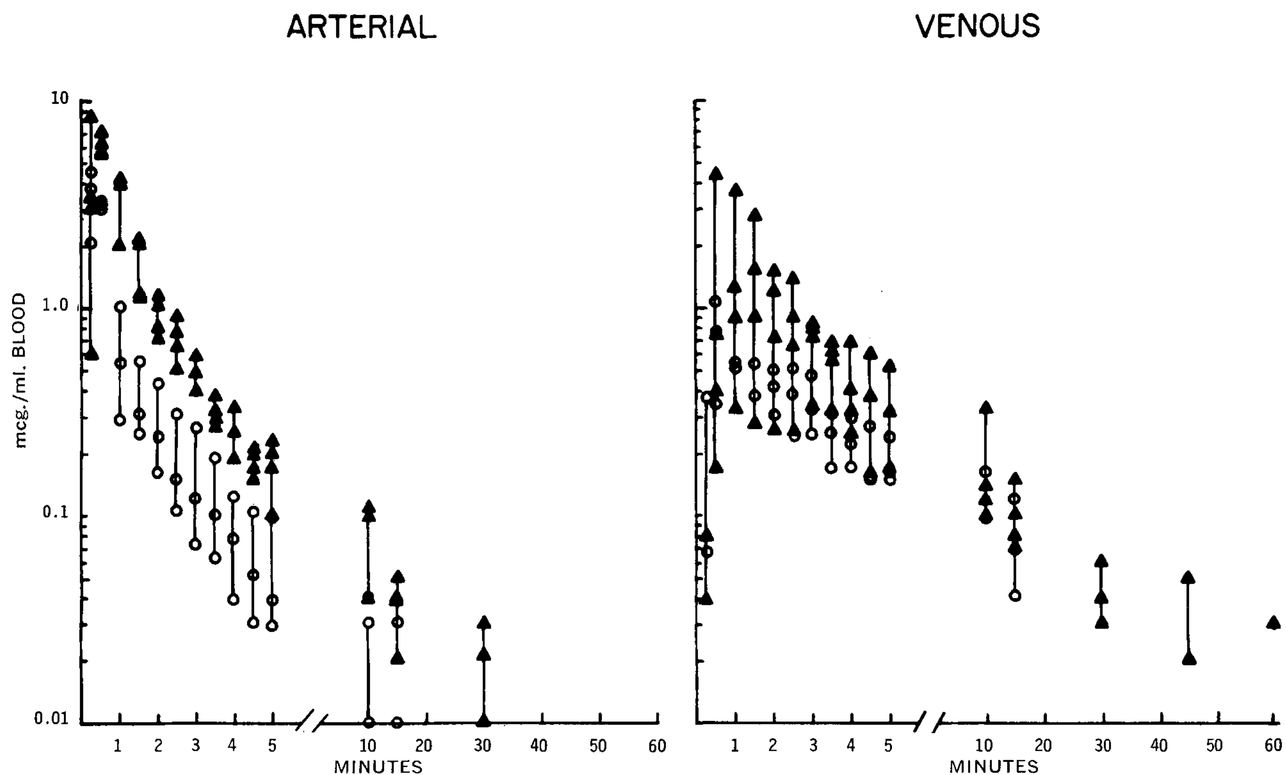

Figure 3—*Dichlorodifluoromethane blood levels in the dog. Five or 10 actuations of an aerosol mixture containing 25% (w/w) of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were administered intratracheally to three or four dogs, one actuation on each successive inspiratory phase of respiration. Each point represents the fluorocarbon blood level obtained from a single dog. Key:* ○, *five actuations; and* ▲, *10 actuations.*

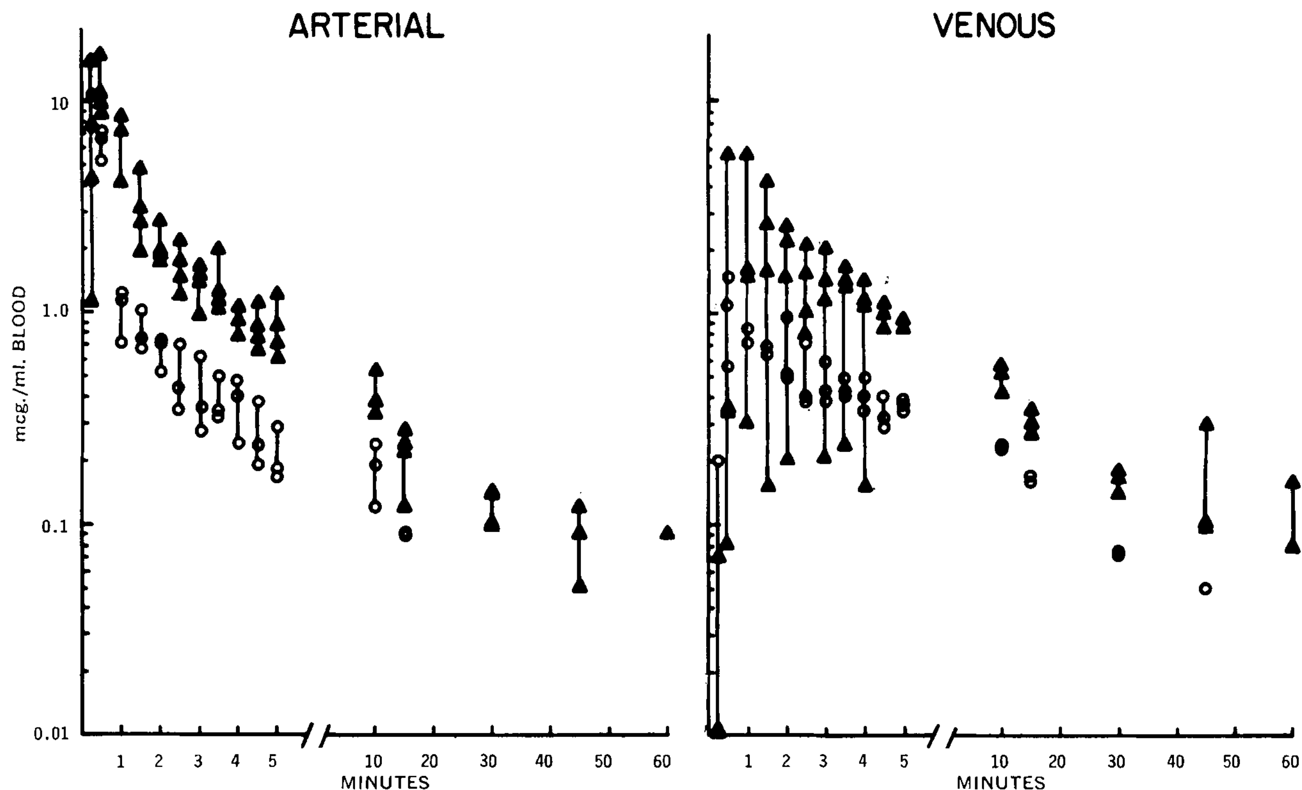

Figure 4—*Trichlorotrifluoroethane blood levels in the dog. Five or 10 actuations of an aerosol mixture containing 25% (w/w) of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were administered intratracheally to three or four dogs, one actuation on each successive inspiratory phase of respiration. Each point represents the fluorocarbon blood level obtained from a single dog. Key:* ○, *five actuations; and* ▲, *10 actuations.*

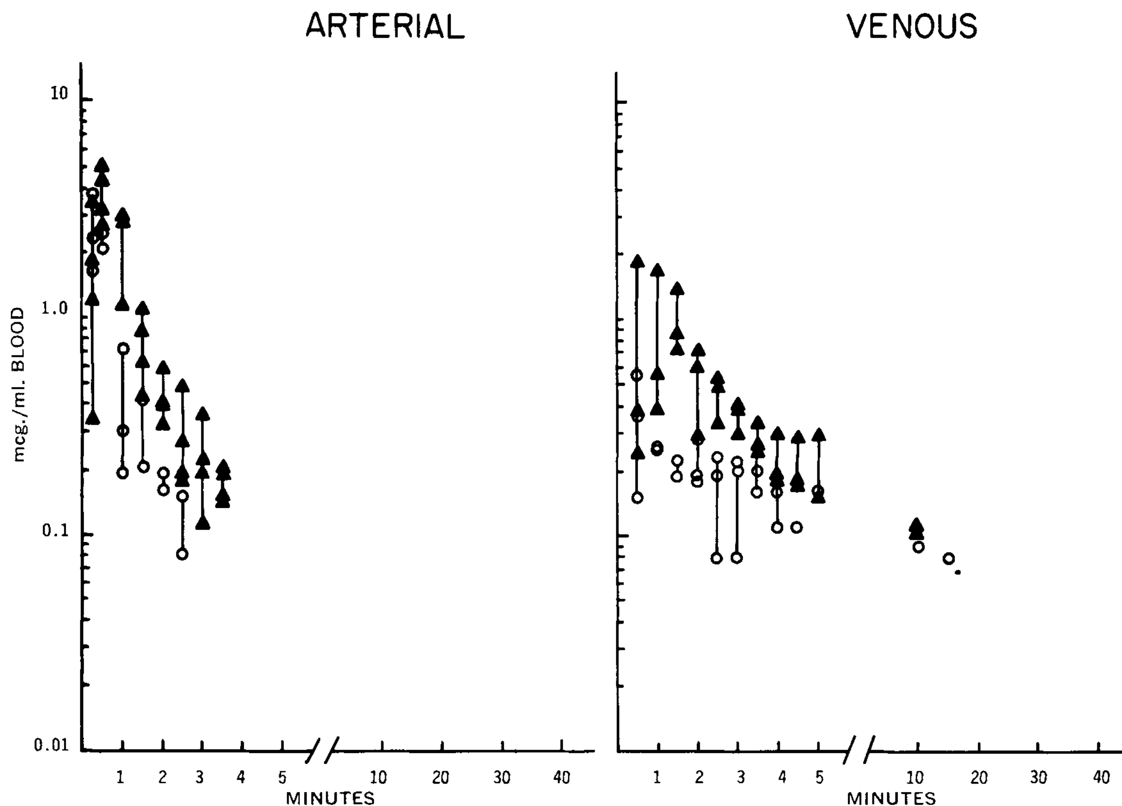

Figure 5—*Dichlorotetrafluoroethane blood levels in the dog. Five or 10 actuations of an aerosol mixture containing 25% (w/w) of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were administered intratracheally to three or four dogs, one actuation on each successive inspiratory phase of respiration. Each point represents the fluorocarbon blood level obtained from a single dog. Key:* ○, *five actuations: and* ▲, *10 actuations.*

Table I—Recovery of Trichloromonofluoromethane, Dichlorodifluoromethane, Trichlorotrifluoroethane, and Dichlorotetrafluoroethane[a]

Fluorocarbon	Percent Recovery
Trichloromonofluoromethane	
ng./ml.	
10	103
20	111
40	91
80	104
160	100
Mean ± *SE*	102 ± 3.2
mcg./ml.	
50	96
100	95
200	102
Mean ± *SE*	98 ± 2.2
Dichlorodifluoromethane	
ng./ml.	
10	108
20	83
40	91
80	103
160	99
Mean ± *SE*	97 ± 2.3
mcg./ml.	
50	98
100	103
200	106
Mean ± *SE*	102 ± 2.3
Trichlorotrifluoroethane	
ng./ml.	
80	91
160	100
320	95
640	100
1280	90
Mean ± *SE*	95 ± 2.1
Dichlorotetrafluoroethane	
ng./ml.	
80	107
160	95
320	106
640	109
1280	87
Mean ± *SE*	101 ± 4.2

[a] Standards were diluted in methanol at dry ice temperature. Dilutions were added to 1.0 ml. heparinized dog blood contained in a vacutainer in ice. Ten milliliters of cold hexane was then added. The vials were mixed well and placed on dry ice prior to analysis. Three microliters of the hexane phase was injected onto the column.

Preparation of Standards—These procedures were designed to handle the more volatile fluorocarbons, *i.e.*, dichlorodifluoromethane and dichlorotetrafluoroethane, as well as trichloromonofluoromethane and trichlorotrifluoroethane.

Dichlorodifluoromethane and dichlorotetrafluoroethane were obtained as liquids from individual gas cylinders by passing the gas through copper tubing immersed in a dry ice–acetone bath. Trichloromonofluoromethane and trichlorotrifluoroethane were obtained as liquids from individual cylinders. The fluorocarbons were poured into a suitable container such as a small glass bottle which was placed on dry ice.

When making all subsequent transfers, all glassware was precooled by bathing in a dry ice–hexane bath. (A dry ice–hexane bath was preferred over a dry ice–acetone bath to prevent contamination of the sample with acetone.)

All dilutions were made by volume. A mean density of 1.62 mg./ml. was calculated for an equal mixture by weight of each fluorocarbon. Trichlorotrifluoroethane freezes at −35° and must be thawed before pipeting. Trichloromonofluoromethane and trichlorotrifluoroethane pipet easily as liquids. Dichlorodifluoromethane and dichlorotetrafluoroethane did not pipet easily in our hands, even with precooled pipets. Thus, dichlorodifluoromethane and dichlorotetrafluoroethane, which had been chilled in a hexane–dry ice bath, were transferred to a small graduated tube (5 or 10 ml.) and then aspirated to the 1.0-ml. mark. The tube was then made to volume (5 or 10 ml.) with precooled hexane. Once diluted in hexane, further dilutions were made using prechilled pipets. Dilutions can be made in the range of 10–1000 ng. fluorocarbon/ml. hexane.

Table II—Peak Blood Levels of Fluorocarbons in Dogs after Aerosol Administration[a]

Fluorocarbon	Peak Level, mcg./ml. 10 Actuations	Peak Level, mcg./ml. 5 Actuations	Peak Arterial Level as Percent of Administered Dose[b] 10 Actuations	Peak Arterial Level as Percent of Administered Dose[b] 5 Actuations
Trichloromonofluoromethane				
Arterial	22.3 ± 1.0	13.2 ± 1.4	15.9	8.89
Venous	6.22 ± 2.6	2.45 ± 0.29		
Dichlorodifluoromethane				
Arterial	6.17 ± 0.38	3.16 ± 0.06	4.41	4.51
Venous	1.54 ± 0.84	0.56 ± 0.04		
Trichlorotrifluoroethane				
Arterial	11.56 ± 1.78	6.43 ± 0.61	8.26	9.19
Venous	2.96 ± 1.40	0.79 ± 0.06		
Dichlorotetrafluoroethane				
Arterial	3.80 ± 0.52	2.32 ± 0.12	2.71	3.31
Venous	0.87 ± 0.41	0.26 ± 0[c]		

[a] Five or ten actuations of an aerosol mixture containing 25% (w/w) of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were administered to three or four dogs, respectively. One actuation delivered 16.8 mg. of each fluorocarbon. The results are expressed as the mean ± *SE* of the peak fluorocarbon blood level determined for three or four dogs. [b] Calculated for a 15-kg. dog with 8% of body weight as blood volume. [c] Mean ± *SE* from two dogs.

Recovery of Fluorocarbons from Dog Blood—The fluorocarbons trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane were diluted volumetrically in methanol, taking similar precautions as described in the preparation of standards. Dilutions were made so that 0.1 ml. of methanol solution contained 10–1000 ng. fluorocarbon. Higher dilutions can be used if desired. Vacutainers (5-ml. draw, containing 12.5 mg. potassium oxalate) were filled with 1.0 ml. heparinized whole blood (pooled from mongrel dogs) by injection through the rubber stopper. [A 1.0-ml. syringe fitted with an 18-gauge, 2.54-cm. (1-in.) needle can be used.] The vacutainer was placed on ice to cool. Aliquots (0.1 ml.) of the methanolic fluorocarbon dilutions were injected (*via* syringe) through the rubber stopper of the vacutainer and mixed with the blood.

Methanol was used to prepare standards for spiking blood so that a single-phase system was obtained. The volume of methanol must be kept to a minimum since larger amounts of methanol will yield a peak on the gas chromatograph at a retention time similar to that of dichlorodifluoromethane.

Two or four milliliters of ice-cold hexane was injected into the vacutainer, and the contents of the tube were mixed well. The sealed vacutainer was then placed in a test tube rack in dry ice. Samples were stable for up to 1 week when stored in dry ice in an upright position.

Fluorocarbons in the hexane phase were assayed as follows. The cap of the vacutainer (which was in dry ice) was removed. An aliquot (3 μl.) of the hexane phase was removed and injected into the gas chromatograph. Recovery of the fluorocarbons was calculated by comparing the peak heights of the hexane-extracted blood samples to standards made up directly in hexane.

RESULTS

The "on-column" lower limit of detection was: trichloromonofluoromethane, 10 pg.; trichlorotrifluoroethane, 120 pg.; dichloro-

difluoromethane, 30 pg.; and dichlorotetrafluoroethane, 240 pg. The sensitivity for the fluorocarbons varies according to the type of electron detector used. The standard curves were constructed by plotting peak heights *versus* concentration. A linear relationship was found for all standards. Recovery of fluorocarbons by hexane extraction of whole blood was virtually 100% (Table I). Trichloromonofluoromethane and dichlorodifluoromethane were extracted equally well at high (micrograms per milliliter) and low (nanograms per milliliter) concentrations.

No internal standard was necessary in this assay. Since the fluorocarbons have high volatility at room temperature, all aliquots of the hexane extract were removed after the solutions had been cooled to dry ice temperature. The precision of the assay and the linearity of the standard curves demonstrated that these propellants were measured with reproducibility and accuracy.

The arterial and venous blood levels in the dog following five or 10 actuations of a pressurized aerosol containing a 25% (w/w) mixture of each fluorocarbon are shown in Figs. 2–5. Arterial fluorocarbon concentrations peaked at 30 sec. after the first actuation of the aerosol. At this time interval, the entire dose (10 actuations) had been administered. Venous fluorocarbon levels were more variable than arterial blood levels. Fluorocarbon concentrations in venous blood peaked at 1.5–2 min. after the first actuation. Venous levels of fluorocarbons declined at rates comparable to those observed in arterial blood. The circulation time as well as mixing of blood *via* arterial-venous shunts may account for the greater variability in the venous data.

The arterial and venous blood concentration–time curves did not fit a single-exponential or biexponential curve. Therefore, a single half-life ($t_{1/2}$) for each fluorocarbon could not be calculated. The data in Figs. 2–5 show that the blood levels of each fluorocarbon declined rapidly, reaching one-half the peak arterial blood level in 0.5 min. or less. Dichlorotetrafluoroethane declined most rapidly followed by trichlorotrifluoroethane, dichlorodifluoromethane, and trichloromonofluoromethane, respectively. Peak arterial levels of each fluorocarbon were found to be 4–5 times that of the peak venous levels (Table II). After the first 2 min., venous levels approximated the arterial levels. Furthermore, the peak arterial levels accounted for only a small percent of the total administered dose. For example, the peak arterial concentration of trichloromonofluoromethane accounted for at most 15.9% of the dose, whereas the peak arterial concentration of dichlorodifluoromethane accounted for only 4.4% of the dose.

DISCUSSION

In earlier attempts by the authors at the assay of fluorocarbon propellants, 1–2 μl. of whole heparinized blood was injected directly on the column. Other groups (5, 6) also used this approach. The extraction of fluorocarbons directly into hexane enables the use of a nonaqueous solution for injection onto the column. Results using injections of whole blood or hexane-extracted samples were essentially similar. The latter method precludes the necessity of cleaning the column at frequent intervals.

Although equal amounts of fluorocarbons (16.8 mg. each of trichloromonofluoromethane, dichlorodifluoromethane, trichlorotrifluoroethane, and dichlorotetrafluoroethane) were administered simultaneously, the arterial peak levels for each fluorocarbon were different and accounted for only a small fraction of the total dose administered. The amount of fluorocarbons absorbed appeared to be related to the physical properties of the individual fluorocarbons.

Dollery *et al.* (5) and Paterson *et al.* (6) reported trichloromonofluoromethane in venous blood samples of humans after administration of a commercial aerosol preparation. The method of Dollery *et al.* (5) did not distinguish between trichloromonofluoromethane and trichlorotrifluoroethane and did not quantitate the more volatile dichlorodifluoromethane and dichlorotetrafluoroethane. Paterson *et al.* (6) were able to separate these fluorocarbons, but they did not report blood levels of dichlorodifluoromethane and dichlorotetrafluoroethane.

The methods used in the studies reported here are extremely sensitive for the measurement of nanogram concentrations of various fluorocarbons in the blood. Furthermore, each fluorocarbon can be separated and quantitated simultaneously. The ease with which hexane-extracted samples can be collected and stored should allow for the use of this method in clinical and laboratory investigations.

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