Academic, New York, N.Y., 1964, pp. 183–203.
(10) G. Deffenu, L. Pegrassi, and B. Lumachi, *J. Pharm. Pharmacol.*, **18**, 135(1966).
(11) "Organic Syntheses," coll. vol. II, A. H. Blatt, Ed., Wiley, New York, N.Y., 1961, pp. 580–583.
(12) A. I. Vogel, in "A Text-Book of Practical Organic Chemistry," 3rd ed., Longmans, Green & Co. Ltd., London, England, 1959, p. 175.
(13) *Ibid.*, p. 176.
(14) O. Hinsberg, *Chem. Ber.*, **43**, 654(1910).
(15) J. Schnekenburger, *Arch. Pharm.*, **297**, 734(1964).
(16) *Ibid.*, **298**, 4(1965).
(17) W. R. Thompson, *Bacteriol. Rev.*, **11**, 115(1947).
(18) R. K. S. Lim, in "The Salicylates: A Critical Bibliographic Review," M. J. H. Smith and P. K. Smith, Eds., Interscience, New York, N.Y., 1966, p. 155.
(19) I. Langmuir, *J. Amer. Chem. Soc.*, **41**, 1543(1919).
(20) O. Hinsberg, *J. Prakt. Chem.*, **93**, 302(1916).
(21) W. Hückel, *Z. Elektrochem.*, **34**, 430(1928).
(22) H. G. Grimm, *ibid.*, **31**, 474(1925).
(23) *Ibid.*, **34**, 430(1928).
(24) H. Erlenmeyer, *Bull. Soc. Chim. Biol.*, **30**, 792(1948).
(25) H. Erlenmeyer and E. Berger, *Biochem. Z.*, **252**, 22(1932).
(26) H. Erlenmeyer, E. Berger, and M. Leo, *Helv. Chim. Acta*, **16**, 733(1933).
(27) H. L. Friedman, in "Symposium on Chemical-Biological Correlation," National Academy of Sciences–National Research Council, Publ. No. 206, Washington, D.C., 1951, p. 295.
(28) J. R. Vaughan, G. W. Anderson, R. C. Clapp, J. H. Clark, J. P. English, K. L. Howard, H. W. Marson, L. H. Sutherland, and J. J. Denton, *J. Org. Chem.*, **14**, 228(1949).
(29) V. B. Schatz, in "Medicinal Chemistry," 2nd ed., A. Berger, Ed., Interscience, New York, N.Y., 1960, p. 72.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 29, 1974, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143*

Accepted for publication October 11, 1974.

* Present address: Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46206

ˣ To whom inquiries should be directed.

Fluorocarbon Aerosol Propellants IV: Pharmacokinetics of Trichloromonofluoromethane following Single and Multiple Dosing in Dogs

SARFARAZ NIAZI and WIN L. CHIOU ˣ

Abstract □ An intravenous dosage form of trichloromonofluoromethane, a common aerosol propellant, was formulated in polyethylene glycol 400 for single and multiple dosing to unanesthetized dogs. A three-compartment open model was proposed for disposition of this compound in dogs with average half-lives of 3.2, 16, and 93 min for three disposition phases. This finding is contrary to several reports where blood levels were monitored for shorter periods. A computer analysis of tissue compartment distribution following a single dose showed that about 2 hr was required to achieve pseudodistribution equilibration, following which more than 90% of the dose remaining in the body was retained in tissue compartments. Pulmonary clearance and volumes of distribution were calculated considering first-pass effect through the lung. The volume of distribution was approximately six times the body weight in terms of blood concentrations, and about 30% of the propellant was cleared from blood passing through the lung in each cycle. Disposition of propellant followed dose-independent kinetics after multiple dosing, and accumulation in tissues continued for a much longer period, resulting in high tissue compartment levels.

Keyphrases □ Trichloromonofluoromethane—pharmacokinetics following single and multiple dosing, dogs □ Fluorocarbon aerosol propellants—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Aerosols—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Propellants—pharmacokinetics of trichloromonofluoromethane following single and multiple dosing, dogs □ Pharmacokinetics—trichloromonofluoromethane, single and multiple doses, dogs

The volatile fluorocarbons have been widely used as aerosol propellants in commercial aerosol packages in this country. Although these compounds have been generally considered nontoxic and inert, several studies (1–3) have claimed that a variable degree of cardiac damage can be caused following their inhalation. An often used argument in favor of low toxicity of fluorocarbons is that these compounds are not absorbed to any significant extent when inhaled from a commercial aerosol product and that the small fraction absorbed is eliminated very fast from the body, decreasing the possibility of any toxic reaction (4–6). Unfortunately, the conclusions drawn from most previous studies monitoring blood levels of fluorocarbons following their inhalation from commercial aerosol products were not based on sound pharmacokinetic principles and do not reflect the true disposition pattern of these compounds as was recently discussed (7).

The objective of the study reported here was to demonstrate that, contrary to the established belief, trichloromonofluoromethane, one of the most commonly used fluorocarbon propellants, has a longer biological half-life than previously thought and under-

goes extensive distribution in the body, an observation that might have an important bearing on the toxicity profile of this compound.

EXPERIMENTAL

Materials—Trichloromonofluoromethane[1] and polyethylene glycol 400[2] were obtained from commercial sources. A medical-grade silicone tubing[3] [0.3 cm (0.125 in.) i.d.] was used with 18–20-gauge cannulas[4] for venous cannulation. The infusion of solution was performed using a constant-rate infusion pump[5].

Formulation of Intravenous Dosage Form—Sterile solutions of fluorocarbons for intravenous infusion were prepared in polyethylene glycol 400, which was sterilized by heating at 100° for about 1 hr over a hot plate. The serum bottles (50-ml capacity) in which the solution was stored were also sterilized by heating at 110° for about 2 hr. After cooling, polyethylene glycol 400 was transferred to almost fill a preweighed, empty, sterile serum bottle, which was then quickly sealed with a lacquer-coated rubber stopper and an aluminum seal with the help of a manual crimper. The bottle was reweighed to determine the weight of polyethylene glycol transferred. The volume of polyethylene glycol was calculated through the density factor of 1.125 (8).

For the preparation of a solution of trichloromonofluoromethane, which exists in liquid form at refrigerator temperature (5°), 0.1–0.5 ml was transferred to the serum bottle using a precooled hypodermic syringe fitted with a 23-gauge needle. Caution was observed to avoid contact of the needle with the polyethylene glycol in the bottle. The bottle was then rotated gently for 15 min to dissolve the fluorocarbon and evenly disperse it. At the end of 15 min, a preweighed 25-gauge hypodermic needle was inserted just below the surface of the rubber stopper to release any excessive pressure and the bottle was reweighed before removing the needle. The weight of the fluorocarbon dissolved was calculated by subtracting the weight of the empty bottle, the polyethylene glycol 400, and the needle.

Analysis of Polyethylene Glycol Solutions—The analysis of the intravenous solutions was performed by transferring a small volume of solution (5–7 drops), using a hypodermic syringe fitted with an 18-gauge needle, to a preweighed and sealed serum bottle (15-ml capacity). The bottle was then reweighed to determine the exact weight of the solution transferred (normally 50–100 mg). The bottles were then rotated in all directions to spread the solution on the wall of the bottles. The bottles were then shaken in a vortex shaker for 10 min to allow complete equilibration between the liquid polyethylene glycol and the air phase.

Although the ratio of the air to liquid phase was very high, such as 300, for 0.05 ml of liquid in a 15-ml capacity bottle, the liquid phase may not be completely depleted of the fluorocarbon upon equilibration. For example, the partition coefficient between polyethylene glycol 400 and air was approximately 11 for trichloromonofluoromethane, which could result in about 3.6% underestimation of the content; proper correction was necessary. The concentration of fluorocarbon in the air phase was analyzed either by directly injecting a small sample of air from the bottle using a gastight syringe on a GC column or by first diluting 100 μl of the air phase in another 15-ml capacity bottle and then injecting a proper volume on the column. The concentration of trichloromonofluoromethane in the polyethylene glycol solution can easily be obtained using the following relationship:

$$C_{peg}\,(\text{mg/ml}) = \frac{C_a V_a D}{W_{peg}} C_a P \qquad \text{(Eq. 1)}$$

where C_a is the concentration of fluorocarbon in the air phase in milligrams per milliliter in the original bottle (if any dilutions are made), V_a is the volume of the air phase (capacity of the bottle, milliliters), D is the density of polyethylene glycol in grams per milliliter, W_{peg} is the weight of polyethylene glycol transferred to the bottle in grams, and P is the partition coefficient between

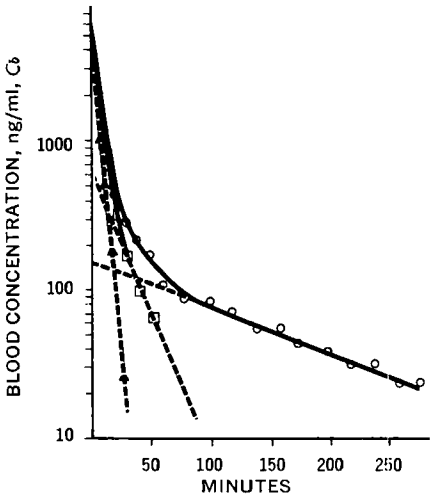


Figure 1—*Triexponential blood concentration decay following intravenous administration of 128.5 mg of trichloromonofluoromethane in 6.6 ml of polyethylene glycol 400 to a dog weighing 19.5 kg.* $C_b = 6000e^{-0.19800t} + 625e^{-0.04531t} + 152e^{-0.00693t}$.

polyethylene glycol 400 and air for trichloromonofluoromethane. Equation 1 is based on the assumption that the dilute solution of the propellant in polyethylene glycol 400 would not significantly affect the density of the polyethylene glycol solution.

The validity of this method of analysis was confirmed by almost perfect agreement between the values obtained gravimetrically (where the weight of fluorocarbon added was known) and those obtained by the described method.

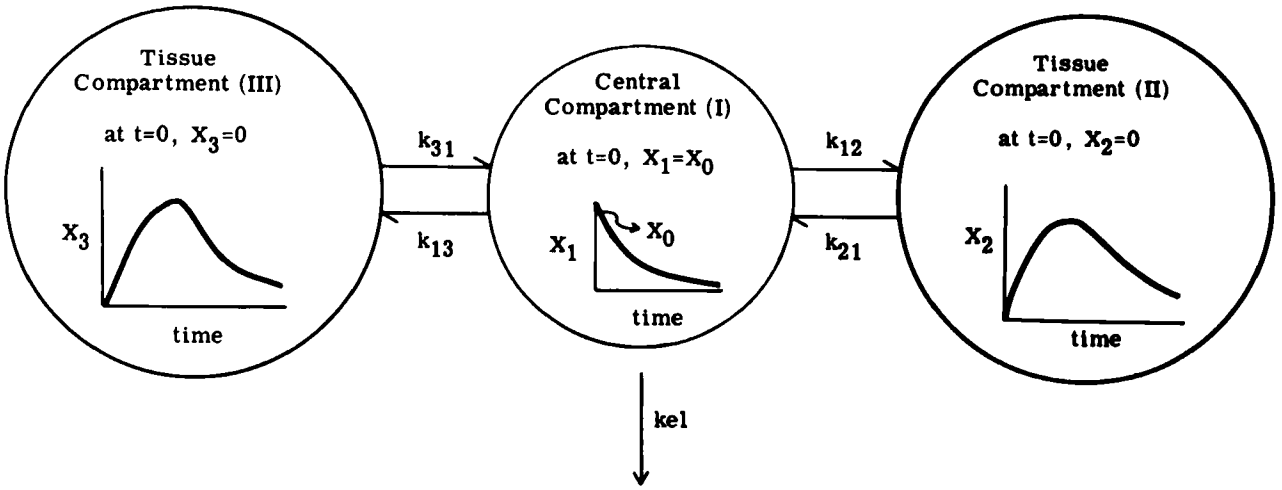
The concentration of trichloromonofluoromethane solutions prepared ranged from 7 to 36 mg/ml. A duplicate analysis in all instances showed a high degree of reproducibility. The analysis of these solutions was also performed just prior to their infusion to account for any loss during overnight storage. A freshly prepared solution (kept overnight) of fluorocarbon in polyethylene glycol was used for each study.

Infusion of Polyethylene Glycol Solutions—The solution stored in the 50-ml serum bottle was withdrawn in a 20- or 50-ml capacity glass syringe using a 16-gauge needle by applying a positive pressure inside the bottle by injecting 20–50 ml of air in the bottle and then slowly filling the syringe, avoiding the entry of air in the syringe. These precautions were necessary to minimize the loss of the fluorocarbon to the atmosphere during the transfer. The glass syringe was then connected to silicone tubing through a three-way stopcock and assembled in a constant-rate infusion pump.

Animal Procedures—Four male, mongrel, unanesthetized, conditioned dogs, 16.8–20 kg, were used. They were fasted for 16–18 hr prior to the experiments, and food and water were withheld during the experiments. The cephalic veins of the forelegs were cannulated using an 18-gauge cannula to which a three-way stopcock was attached. The syringe containing the fluorocarbon solution was connected to the cannula through silicone tubing. The solution was infused over 3 min at a constant rate of 2.2 ml/min for the single-dose study and for 2 min at a rate of 2.5 ml/min for the multiple-dose study. At the end of the infusion period, the cannula was flushed with 5 ml of heparinized sterile normal saline.

The blood samples (0.5–3.0 ml) during the single-dose study were collected from the other leg at 0, 1, 2, 3, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 100 min and then up to 300 min at every 20 min. The zero time for sampling was assigned when the infusion was completed. A blank blood sample was obtained before the solution was infused to study the partition coefficients. The cannula through which the solution was infused was removed as soon as possible after the infusion was complete. In the case of multiple dosing, the cannula was left intact until the last dose was infused. The samples obtained during the multiple-dose study were less frequent except for the last dose, after which a pattern similar to that described for the single-dose study was followed. Every time a blood sample was withdrawn, the cannula was flushed with heparinized normal saline to avoid clotting.

[1] Supplied by E. I. duPont deNemours & Co., Wilmington, Del.
[2] Union Carbide Chemical Co., New York, N.Y.
[3] Dow Corning Corp., Midland, Mich.
[4] Medicut, Aloe Medical, St. Louis, Mo.
[5] Harvard Apparatus Co., Millis, Mass.



Scheme I—A three-compartment disposition model for trichloromonofluoromethane in dogs

During all studies, an adequate room ventilation was maintained and care was observed not to excite the animal since the cardiac output and breathing rate can affect the results significantly. No study was performed if the animal got highly excited during the process of cannulation. At least 1 week was allowed between studies in the same animal. A duplicate study was performed on Dog 2976.

Analysis of Trichloromonofluoromethane in Blood—Approximate volumes of blood samples, ranging from 0.5 to 3.0 ml, were quickly transferred using a 23-gauge needle to sealed preweighed and heparinized serum bottles of various capacities; a 27-gauge needle had been inserted to release the excessive pressure buildup due to the added volume of the blood. Both needles were removed quickly after the blood samples were transferred. The serum bottles were then reweighed to determine the weight of blood added to each bottle, which was then converted to volume using the experimentally determined density factor of 1.04. The blood samples were allowed to equilibrate in the sealed serum bottle with the air phase, which was then analyzed. Then the concentration of the blood samples was determined using the following relationship (9):

$$C_b = \frac{C_h(V_t - V_b)}{V_b} + C_hP \qquad \text{(Eq. 2)}$$

where C_b and C_h are the concentrations in the blood sample transferred to the serum bottle and the head space in the serum bottle with which it equilibrates, respectively; V_t and V_b are the volumes of the serum bottle and the blood sample transferred, respectively; and P is the partition coefficient between blood and air. Reference 9 should be consulted for a detailed description of the procedure.

The analyses were performed as soon as possible after withdrawing the blood samples to avoid any loss during storage. Duplicate analyses were performed on each sample and a high degree of reproducibility was always achieved.

RESULTS AND DISCUSSION

Dosage Form and Route of Administration—In most previous studies measuring blood concentrations of fluorocarbons following their inhalation, a large degree of variation was inevitably seen for several reasons. First, the uptake of gases largely depends on cardiac output and ventilation (10), both of which depend on such factors as levels of excitement, blood carbon dioxide tension, and pathological conditions. Second, the uptake of gases is further complicated by the rebreathing from the residual volumes in the lungs and from the atmosphere. For example, Shargel and Koss (11) found variations of more than 50-fold in blood concentrations of fluorocarbons following inhalation of equal doses in dogs. Such variations were also reported by Azar *et al.* (12) and were attributed to the reasons listed here.

One means of curtailing such large variations is to use a dosage form other than inhalations. For example, a solution of fluorocarbon in corn oil was used for oral administration to rats (13). How-

Table I—Pharmacokinetic Parameters of Trichloromonofluoromethane from Five Studies

Parameter[a]	Dog 3287	Dog 3288	Dog 3457	Dog 2976a	Dog 2976b
b_1	0.239	0.198	0.252	0.193	0.222
b_2	0.0504	0.0433	0.0590	0.047	0.0308
b_3	0.0084	0.00693	0.00825	0.00753	0.00660
C_1	0.714	0.885	0.81	0.573	0.897
C_2	0.220	0.0922	0.176	0.382	0.0849
C_3	0.0612	0.0224	0.00796	0.0446	0.0179
C_{12}	−0.330	−0.248	−0.306	−0.331	−0.348
C_{22}	0.333	0.231	0.301	0.309	0.319
C_{32}	0.049	0.0169	0.00555	0.0216	0.0287
C_{13}	−0.129	−0.183	−0.590	−0.0665	−0.125
C_{23}	−0.255	−0.107	−0.0620	−0.218	−0.115
C_{33}	0.384	0.290	0.121	0.284	0.239
k_{e1}	0.0679	0.102	0.139	0.0592	0.105
k_{21}	0.100	0.0589	0.0936	0.108	0.0487
k_{31}	0.0148	0.00993	0.00940	0.0108	0.00882
k_{13}	0.0404	0.0388	0.0175	0.021	0.0296
k_{12}	0.0742	0.0390	0.0594	0.0488	0.0673
D^0,[b] mg	209	128	241	116	49.2
AUC^∞, μg/ml, min	144	66.6	175	132	49.8

[a] The units for all b and k values are minutes^{-1}. [b] $X_0 = (1 - f)D^0$, where $(1 - f)$ is the fraction eliminated in the first pass through the lungs.

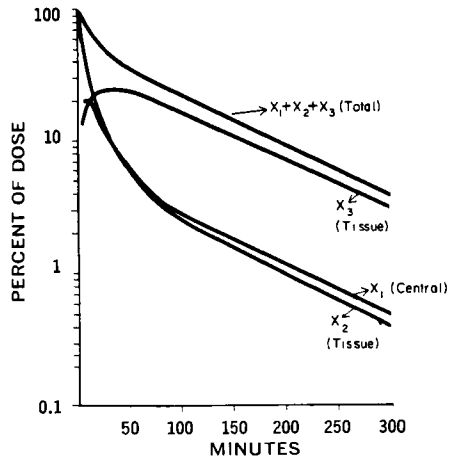


Figure 2—*Percent of absorbed trichloromonofluoromethane remaining in various body compartments based on a typical study (Dog 3287).*

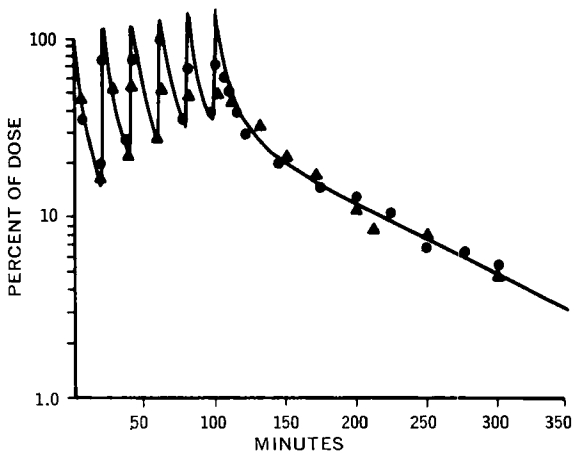


Figure 4—*Percent of absorbed dose remaining in the central compartment following six intravenous administrations of 43 mg (▲, Dog 3287) or 62 mg (●, Dog 3288) of trichloromonofluoromethane every 20 min. The solid curve represents the theoretical values based on a typical set of pharmacokinetic parameters obtained from a single-dose study (Dog 3287).*

ever, the biological availability problems in this case can complicate the situation. An intravenous dosage form offers the greatest advantages of control and ease of administration. Unfortunately, trichloromonofluoromethane presents the problem of extremely low solubility in water-miscible liquids that can be used as vehicles in intravenous dosage forms.

The choice of polyethylene glycol 400 was arrived at by the process of elimination of solvents that will have adequate solubility, show low toxicity when administered intravenously, and be stable to the sterilization procedure. This vehicle was previously used to formulate injections (14, 15). This vehicle, however, does not represent an ideal choice in view of a recent report showing that the administration of polyethylene glycol 400 increased the blood pressure response to 10 μg of epinephrine by an average of 22 mm Hg (27% increase over control). The polyethylene glycol 400 itself did not show any affect, but the response enhancement to epinephrine was dose dependent and lasted for about 1 hr following administration of a minimum dose producing an enhancement to epinephrine response. This observation, however, would not affect the utility of this dosage form in the present study where the main objective was to study the disposition of fluorocarbons rather than their toxic manifestations, which might possibly be complicated if polyethylene glycol 400 is used as a dosage vehicle. The amount of the fluorocarbon administered in this study was close to the total fluorocarbons that may be encountered clinically after inhalation.

Pharmacokinetic Model—The blood concentrations (venous) obtained at different time intervals following intravenous dosing of the fluorocarbon were plotted on semilogarithmic paper; the midpoint of infusion was assigned as zero time and a smooth curve was drawn through these points. A typical example is shown in Fig. 1. The feathering method (17) was then employed to isolate different exponents. In all instances, the blood concentration–time curves were characterized by a triexponential equation. A three-compartment open model for trichloromonofluoromethane is depicted in Scheme I. The two compartments besides the central compartment can be described as the group of tissues with distinct affinity and capacity (17) for trichloromonofluoromethane. The difference in affinity may be due to the solubility characteristics or to specific interactions such as protein binding or other complexations. A compartment can also be distinguished based on the blood supply to the tissues such as a vessel-rich group or a vessel-poor group (18).

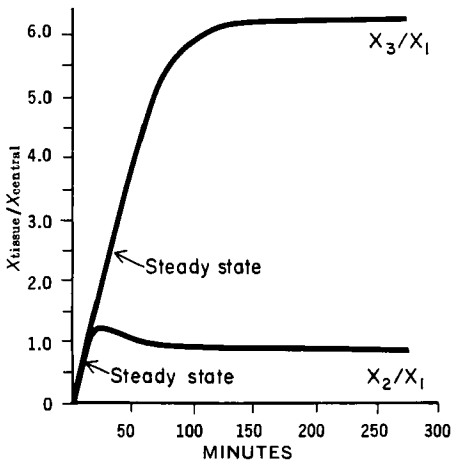


Figure 3—*Comparison of the relative amount of trichloromonofluoromethane remaining in tissue and central compartments based on a typical study (Dog 3287).*

For further analysis of this model, to calculate various rate constants as described in Scheme I, a computer program was generated based on the differential equation solutions described by Rescigno and Segre (17).

The fraction of dose remaining in various pharmacokinetic compartments can be described in terms of hybrid constants:

$$X_1/X_0 = C_1e^{-b_1t} + C_2e^{-b_2t} + C_3e^{-b_3t} \quad \text{(Eq. 3)}$$

$$X_2/X_0 = C_{12}e^{-b_1t} + C_{22}e^{-b_2t} + C_{32}e^{-b_3t} \quad \text{(Eq. 4)}$$

$$X_3/X_0 = C_{13}e^{-b_1t} + C_{23}e^{-b_2t} + C_{33}e^{-b_3t} \quad \text{(Eq. 5)}$$

where X_1, X_2, and X_3 are the amounts of fluorocarbon present in different compartments, and X_0 is the dose, which in the model proposed is the dose remaining in the body after the first pass through the lungs as described later.

The described pharmacokinetic parameters determined experimentally for trichloromonofluoromethane are reported in Table I.

Volume of Distribution and Pulmonary Clearance—The apparent volume of distribution, V_d, of a drug after reaching a pseudodistribution–equilibrium state following intravenous administration has been defined as (19):

$$(V_d)_{b_3} = \frac{D^0}{b_3 \times AUC} \quad \text{(Eq. 6)}$$

where AUC is the area under the blood concentration–time curve from zero to infinity, b_3 is the first-order disposition constant in the terminal phase, and D^0 is the dose administered.

Equation 6, however, cannot be used for the present situation where trichloromonofluoromethane was administered in the venous blood and where, therefore, a significant fraction may be eliminated from the lungs before the concentration in the venous blood is monitored, resulting in a serious overestimate of the volume of distribution if D^0 is assumed to be equivalent to the dose absorbed. Therefore, a correction factor is necessary to account for the fraction eliminated in the first pass through the lungs:

Table II—Volumes of Distribution and Clearance of Trichloromonofluoromethane

Dog	$(V_d)_{b_3}$, liters	V_c, liters	PC, liters/min	$(1 - f)$, %
3287	116.28	14.44	0.98	32.57
3288	169.33	11.51	1.17	39.11
3457	114.23	6.75	0.94	31.41
2976a	90.32	11.49	0.68	22.67
2976b	112.56	7.04	0.74	24.76
Mean ± *SEM*	120.54 ± 13.07	10.25 ± 1.47	0.90 ± 0.10	30.09 ± 2.95

$$(V_d)_{b_3} = \frac{f \times D^0}{b_3 \times AUC} \qquad \text{(Eq. 7)}$$

where f is the fraction of the administered dose retained in the body following the first pass through the lung. The fraction eliminated by the first pass can be calculated by the following equation in analogy to one proposed for the first-pass effect following oral dosing (20):

$$\text{fraction eliminated} = (1 - f)\frac{PC}{PFR} \qquad \text{(Eq. 8)}$$

where PFR is the pulmonary flow rate, which is almost equivalent to the cardiac output (an average value of 3 liters was used as reported in Ref. 21), and PC is the pulmonary clearance:

$$PC = (V_d)_{b_3} \times b_3 \qquad \text{(Eq. 9)}$$

Substitution of Eq. 9 into Eq. 8 and elimination of f from Eq. 7 lead to the following equation (22):

$$(V_d)_{b_3} = \frac{PFR \times D^0}{b_3(AUC \times PFR + D^0)} \qquad \text{(Eq. 10)}$$

This equation, however, assumes that the pulmonary clearance is the only route of elimination for the compound. The fluorocarbon studied in this investigation was shown to be primarily eliminated intact from the lung in rats (23). Therefore, Eq. 6 can be used as an approximation for the determination of the apparent volume of distribution and the pulmonary clearance. The volume of distribution for trichloromonofluoromethane (Table II) was calculated to be approximately six times the average body weight (16.8–20 kg), an indication that this compound is extensively distributed in the body tissues.

The pulmonary clearance values were calculated using Eq. 9 and are also reported in Table II. An average clearance of 900 ml/min corresponds to 30% clearance in each cycle through the lung, and an error of this magnitude would have been involved in the calculations of volumes of distribution if the correction due to the first pulmonary pass effect had not been made.

The volume of the central compartment, from where the actual elimination takes place, can be calculated easily since the clearance from the central compartment should equal the clearance from the body:

$$(V_d)_{b_3} \times b_3 = V_c \times k_{el} \qquad \text{(Eq. 11)}$$

where k_{el} is the elimination rate constant from the central compartment. Table II also shows the values for the volumes of the central compartment. Smaller volumes as compared to the total volumes of distribution indicate retention of a smaller fraction in the central compartment as compared to tissue compartments after the pseudodistribution phase.

Table III—Disposition Half-Lives of Trichloromonofluoromethane

Disposition Phase	Half-Life (Mean ± *SEM*), min (n = 5)
Initial	3.17 ± 0.16[a]
Intermediate	15.70 ± 1.83
Final	92.70 ± 4.39

[a] This value may be slightly different from the true value because the experimental value would be influenced by the duration of the intravenous infusion.

Disposition Half-Lives—Table III shows the half-lives for the three disposition phases of trichloromonofluoromethane. The terminal disposition half-lives reported here are much longer than previously reported (11, 12, 24–26). An obvious reason for this large discrepancy is that the blood concentrations were monitored previously for only short intervals. The longest period involved was about 60–70 min. However, as shown in Fig. 1, it took about 100 min to reach the terminal phase. Consequently, in all of the mentioned studies, the fluorocarbons had not yet equilibrated with the body tissues, resulting in much shorter apparent half-lives due to the distribution to the tissues.

An analysis of the data reported (11) for the disposition of fluorocarbons in dogs shows that during the 10–60-min time interval the half-life of elimination appeared to be about 20 min for trichloromonofluoromethane. The actual value will be lower if the terminal phase is feathered out. This value is in agreement with the value in this study for the intermediate disposition phase. An analogous scrutiny of other reported data (26) shows that the terminal half-life for trichloromonofluoromethane was about 90 min in a human subject, an observation that went unnoticed by these investigators.

The finding that trichloromonofluoromethane shows a multiexponential decay in the blood concentration can be utilized to study the true elimination rate from the body and to evaluate the degree of accumulation in the body following single or multiple exposures.

Retention following Single Intravenous Administration—Following administration of trichloromonofluoromethane through intravenous infusion, the concentration in the blood drops very sharply. Such an observation was also reported for fluorocarbons following their inhalation (25, 26). However, such sharp declines in blood concentrations do not necessarily follow a parallel loss of the drug from the body (27). The amount of the drug remaining in the body (addition of Eqs. 3–5) as a function of time can also be calculated using the following equation (27):

$$\text{fraction remaining in body} = \frac{(C_1/b_1)e^{-b_1 t} + (C_2/b_2)e^{-b_2 t} + (C_3/b_3)e^{-b_3 t}}{(C_1/b_1) + (C_2/b_2) + (C_3/b_3)} \qquad \text{(Eq. 12)}$$

Figure 2 shows a plot of the amount of trichloromonofluoromethane remaining in the body and in various tissue compartments as described earlier (Eqs. 3–5). For example, the amount of trichloromonofluoromethane in the central compartment dropped to about 15% of the absorbed dose (dose after the first pulmonary pass) in 25 min, but about 55% of the absorbed dose was still present in the body.

It is evident that a sharp buildup of the administered dose continues in the tissues until a steady state is reached when the rate of change of the amount present in the tissues equals zero; this steady state is then followed by a decline in the amounts present in the tissue compartments. The ratio of the fraction of the dose in the tissues over that in the central compartment, however, keeps increasing since the rate of loss from the central compartment is greater than from the tissue compartments until the pseudoequilibration stage is reached; then the ratio of the amount in the tissue compartments to the amount in the central compartment remains constant (18). To demonstrate this, a plot was made (Fig. 3) based on the data presented in Fig. 2. It is evident that although the

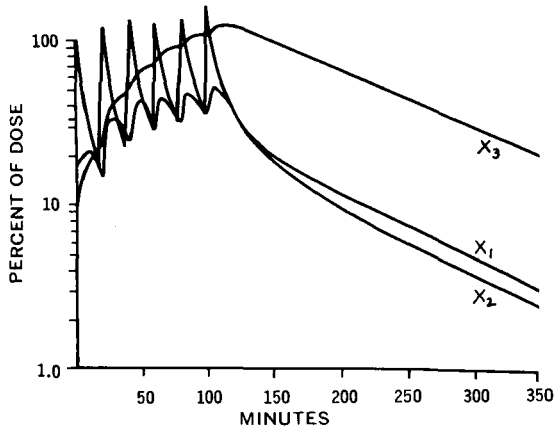


Figure 5—*Computer-generated curves for the distribution of trichloromonofluoromethane in various pharmacokinetic compartments following instantaneous absorption of six doses of 100 units each every 20 min. The pharmacokinetic parameters were obtained from a typical single-dose study (Dog 3287).*

steady state is reached rather quickly, as indicated by arrows in Fig. 3, the ratio of the amount in the tissue compartments over the central compartment keeps increasing until a pseudodistribution–equilibrium is reached. The retention of trichloromonofluoromethane, therefore, continues for a considerable period; for example, in the study reported in Scheme I, it took about 125 min to reach equilibration with the central compartment.

This finding is important because it shows for the first time that the claims made regarding fast elimination of fluorocarbons from the body (12, 25, 26) are probably erroneous and can be misleading in the evaluation of the toxicity of this compound.

In Compartment 2 (X_2), a peak was observed for the ratio of fluorocarbon in the tissue compartment over that in the central compartment. This kind of peak ratio was not observed for Compartment 3 (X_3). This difference can be explained on the basis of the capacity of the two compartments. Compartment 3 shows a much higher capacity for fluorocarbon retention compared to Compartment 2. In the initial phase of distribution, an "apparent supersaturation" of Compartment 2 may result due to the strong driving force from the central compartment because of the higher concentration. This apparent supersaturation might not exist in Compartment 3 because of its higher capacity.

The total amount of the dose retained following pseudodistribution–equilibrium is an important parameter, especially when extrapolating the single-dose study to multiple-dose studies. Table IV reports the retention of trichloromonofluoromethane in the tissue and central compartments after pseudodistribution–equilibrium has been reached. The fraction of the fluorocarbon retained in the tissue compartments compared to the central compartment shows that more than 90% of the total amount present in the body was retained in tissue compartments. This finding clearly shows that a sharp decline in the concentration in the central compartment does not necessarily parallel the decline in the amount in the body, an observation that was not well recognized in most studies reported earlier.

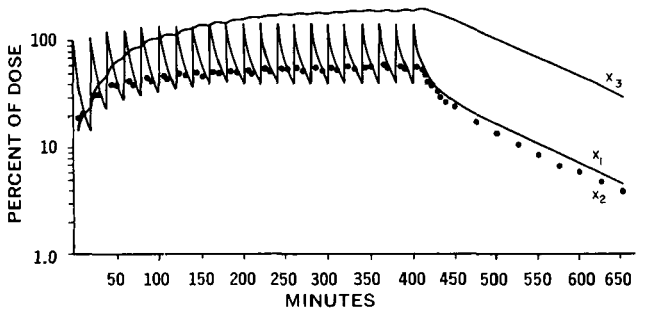


Figure 6—*Computer-generated curves for the distribution of trichloromonofluoromethane in various pharmacokinetic compartments following instantaneous absorption of 21 doses of 100 units each every 20 min. The pharmacokinetic parameters were obtained from a typical single-dose study (Dog 3287).*

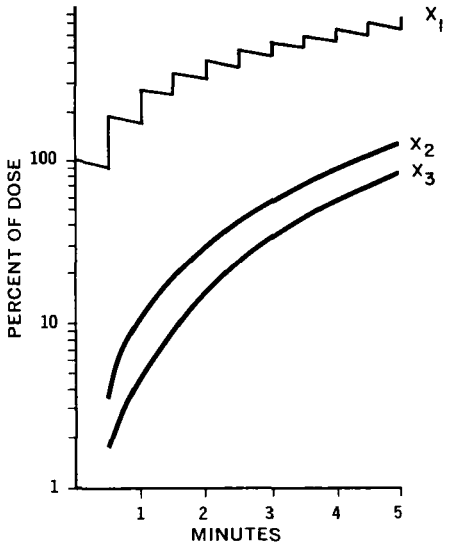


Figure 7—*Computer-generated curves for the distribution of trichloromonofluoromethane in various pharmacokinetic compartments following instantaneous absorption of 11 doses of 100 units each every 30 sec. The pharmacokinetic parameters were obtained from a typical single-dose study (Dog 3287).*

Multiple-Dose Kinetics—The pharmacokinetic parameters calculated following single-dose administration (Table I) can be used to simulate multiple-dose kinetics. A typical study, such as with Dog 3287, was used to generate multiple-dose disposition curves[6]. These curves were then superimposed by the experimental data obtained from two separate studies (Fig. 4). A good agreement between theoretical and experimental values provides the basis for the validity of the premise involved in the generation of multiple-dose curves that each dose decays independently of the amount present in the body or, in other words, that dose-independent kinetics are operative for the disposition of this compound in the dose range studied.

A significant aspect of this study is that it allows the assessment of the accumulation of trichloromonofluoromethane in the tissue compartments during multiple exposures in the frequent use of commercial products containing fluorocarbons as propellants. With fast intravenous infusion of a drug, it has often been shown (28) that a large fluctuation in the concentration in the central compartment is seen which is not necessarily paralleled in the tissue compartments because of the essential nature of the time-dependent process of distribution from the central compartment to the tissue compartments.

Figure 5 shows the calculated amount of trichloromonofluoromethane distributed to various tissue compartments following six multiple doses. The amount remaining in the tissue compartments shows much less fluctuation than is observed in the central compartment (the ordinate is in the logarithmic scale, which reduces the apparent variation). For example, Fig. 5 shows that the amount in Compartment 2 varied 1.5 times between the lowest level before administration and the peak level following the administration of last dose, whereas the amount in the central compartment varied almost four times. Compartment 3 showed the least fluctuation of about 1.13 times. Some correlation between the degree of fluctuation and the nature of the compartment can be drawn based on the data presented earlier regarding the capacity of various tissue compartments. The tissue compartment, which has a higher capacity to retain the fluorocarbon, would be expected to show less fluctuation in its highest and lowest levels during multiple-dose administration since a longer time may be required to equilibrate this compartment with the central compartment.

[6] IBM computer facility at the University of Illinois Medical Center.

Table IV—Average Retention ($\pm SEM$) of Trichloromonofluoromethane at 300 min following Pseudo-Distribution–Equilibrium in Various Compartments

Percent	$X_2 + X_3$ Compartments	X_1 Compartment
Percent of absorbed dose	3.05 ± 0.51	0.31 ± 0.23
Relative percent of total amount in the body	90.8 ± 15.2	9.2 ± 6.8

The magnitude of the problem of accumulation during multiple exposures to trichloromonofluoromethane is well emphasized in this study. For example, the total amount eventually accumulated in the tissues may well exceed the amount administered in one dose. This situation is shown in Fig. 5 where the total amount accumulated in two tissue compartments at the peak just after the last dose was about 1.65 times the dose that was administered every 20 min.

The computer-simulated curve for 21 consecutive doses at intervals of 20 min (Fig. 6) shows similar fluctuations of the amount in the central compartment as was observed for a six-dose study (Fig. 5). The fluctuation in the tissue compartments decreased gradually as the equilibrium was approached, and until then a buildup of trichloromonofluoromethane continued. The magnitude of the buildup would not be apparent if blood concentration alone were used to represent the body levels as was attempted by Dollery *et al.* (26). These investigators administered a mixture of fluorocarbons containing trichloromonofluoromethane by inhalation to humans every 10 min for 6 hr and failed to notice any significant rise in the blood concentration of trichloromonofluoromethane. They attributed the result to the possible lack of accumulation in the body.

The observations made in this study may have some bearing in hazard potential evaluation for professionals who come in frequent contact with aerosol packages containing fluorocarbon propellants.

The total accumulation in the body following multiple dosing depends on such factors as the dose administered and the frequency of administration. An interesting simulation of the accumulation pattern can be made to show the commonly observed excessive inhalation of bronchial aerosols by desperate asthmatic patients. A time interval of 0.5 min was chosen to administer 100 units of trichloromonofluoromethane through lungs in a typical study; the results in Fig. 7 show that in 5 min, during which 10 administrations were made, almost 96% of the trichloromonofluoromethane absorbed was retained in the body. Although a major fraction was present in the central compartment, the amount in the tissue compartments was more than twice the single dose administered. This massive buildup might also occur in youths who inhale aerosol packages to "turn on."

The toxicological aspects of the observations made here can be twofold. First, during excessive inhalation, it is possible that high tissue levels may be reached in spite of the high volatility of trichloromonofluoromethane. The sudden deaths reported following inhalation of aerosol packages can be attributed at least in part to threshold toxic limits, although no such direct and reliable data have been reported. Second, the accumulation following multiple exposures can result in much higher deep tissue compartment levels than are apparent from blood levels. Therefore, the possibility of chronic toxicity cannot be ruled out entirely.

The toxicological implications of the use of aerosol packages containing fluorocarbon propellants and the environmental pollution hazard from these compounds are matters of controversy, but the data presented here warrant against misleading toxicological interpretations that might result in the application of erroneous pharmacokinetic principles.

REFERENCES

(1) F. E. Speizer, R. Doll, and P. Heab, *Brit. Med. J.*, **1**, 335(1968).

(2) V. E. Archer, *ibid.*, **3**, 696(1973).

(3) M. Bass, *J. Amer. Med. Ass.*, **212**, 2075(1970).

(4) G. J. Taylor and W. S. Harris, *J. Clin. Invest.*, **50**, 1546(1971).

(5) G. J. Taylor and W. S. Harris, *J. Amer. Med. Ass.*, **214**, 81(1970).

(6) N. C. Flowers and L. G. Horan, *ibid.*, **219**, 33(1972).

(7) W. L. Chiou, *ibid.*, **227**, 658(1974).

(8) "Remington's Pharmaceutical Sciences," 14th ed., A. Osol *et al.*, Eds., Mack Publishing Co., Easton, Pa., 1970, p. 1355.

(9) W. L. Chiou and S. Niazi, *Res. Commun. Chem. Pathol. Pharmacol.*, **6**, 481(1973).

(10) A. Goldstein, L. Avonow, and S. M. Kalman, "Principles of Drug Action," Harper and Row, New York, N.Y., 1969, p. 323.

(11) L. Shargel and R. Koss, *J. Pharm. Sci.*, **61**, 1445(1972).

(12) A. Azar, H. J. Trochimowicz, J. B. Terrill, and L. S. Mullin, *Amer. Ind. Hyg. Ass. J.*, **34**, 102(1973).

(13) J. B. Terrill, *ibid.*, **33**, 736(1972).

(14) S. Riegelman, W. L. Epstein, and R. J. Dayan, Presented to the Scientific Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, 1962.

(15) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **60**, 1376(1971).

(16) R. D. Heilman, E. W. Bauer, and J. P. DaVanzo, *Toxicol. Appl. Pharmacol.*, **23**, 263(1972).

(17) A. Rescigno and G. Segre, "Drug and Tracer Kinetics," Blaisdell, New York, N.Y., 1966, pp. 57–137.

(18) M. Gibaldi, R. Nagashima, and G. Levy, *J. Pharm. Sci.*, **58**, 193(1969).

(19) D. Perrier and M. Gibaldi, *J. Pharmacokin. Biopharm.*, **1**, 17(1973).

(20) M. Rowland, in "Current Concepts in Pharmaceutical Sciences: Dosage Form Design and Bioavailability," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1973, p. 181.

(21) W. S. Spector, "Handbook of Biological Data," W. B. Saunders, Philadelphia, Pa., 1956.

(22) W. L. Chiou, *Res. Commun. Chem. Pathol. Pharmacol.*, **7**, 679(1974).

(23) P. J. Cox, L. J. King, and D. V. Parke, *Biochem. J.*, **130**, 13p(1972).

(24) D. A. McClure, *Toxicol. Appl. Pharmacol.*, **22**, 221(1972).

(25) J. W. Paterson, M. F. Sudlow, and S. R. Walker, *Lancet*, **2**, 565(1971).

(26) C. T. Dollery, G. H. Draffan, G. H. Davies, F. M. Williams, and M. E. Conolly, *ibid.*, **1**, 1164(1970).

(27) W. L. Chiou, *J. Pharm. Pharmacol.*, **24**, 342(1972).

(28) M. Gibaldi, G. Levy, and H. Weintraub, *Clin. Pharmacol. Ther.*, **12**, 734(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 24, 1974, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication October 11, 1974.

Abstracted in part from a thesis submitted by S. Niazi to the Graduate College, University of Illinois at the Medical Center, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by Food and Drug Administration Grant 1 RO1 FD-00574-01.

[x] To whom inquiries should be directed.