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Fluorocarbon Aerosol Propellants X: Pharmacokinetics of Dichlorotetrafluoroethane in Dogs

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Abstract □ An intravenous dosage form of dichlorotetrafluoroethane, a common fluorocarbon aerosol propellant, was formulated in polyethylene glycol 400 for single dosing to unanesthetized dogs. A three-compartment open model was proposed for the disposition of this compound in dogs, with average half-lives of 1.3, 9.6, and 50.8 min for the three disposition phases. An analysis of tissue compartment distribution following a single dose showed that it took about 2 hr to achieve pseudo-distribution equilibration, following which more than 90% of the propellant remaining in the body was retained in the tissue compartments. Pulmonary clearance and volumes of distribution were calculated considering the first-pass effect through the lungs. The volume of distribution was approximately 10 times the body weight in terms of blood concentration, and about 84% of the propellant was cleared from the blood passing through the lungs in each cycle.

Keyphrases □ Fluorocarbon aerosol propellants—dichlorotetrafluoroethane, tissue compartment, distribution analysis, three-compartment open model, dogs □ Dichlorotetrafluoroethane—tissue compartment distribution analysis, three-compartment open model, dogs □ Aerosol propellants, fluorocarbon—dichlorotetrafluoroethane, tissue compartment distribution analysis, three-compartment open model, dogs □ Pharmacokinetics—dichlorotetrafluoroethane, tissue compartment distribution analysis, three-compartment open model, dogs

The wide use of fluorocarbon aerosol propellants in various household, cosmetic, and medicinal pressurized packages has recently prompted extensive studies on their possible adverse effects. These include effects on the cardiovascular system (1–4), enzyme activities (5), mutation (6), and ozone concentrations in the stratosphere (7). Arguments that have often been presented in favor of the low systemic toxicity of fluorocarbons is that these compounds are not absorbed to any significant extent when inhaled from commercial aerosol products and that the small fraction absorbed is eliminated rapidly from the body, decreasing the possibility of any toxic reaction (8–10).

Unfortunately, the conclusions drawn from most previous studies were not based on sound pharmacokinetic principles and do not reflect the true disposition pattern of these compounds (4, 11).

The objectives of this study were to demonstrate that dichlorotetrafluoroethane, one of the most commonly used fluorocarbon propellants, has a longer biological half-life than previously thought and undergoes extensive distribution in the body, an observation that might have an important bearing on its toxicity profile.

EXPERIMENTAL

Materials—A medical-grade silicone tubing[1] [0.3 cm (0.125 in.) i.d.] was used with 18–20-gauge cannulas[2] for venous cannulation. The infusion of the solution was performed using a constant-rate infusion pump[3].

Intravenous Dosage Form—Sterile solutions of dichlorotetrafluoroethane[4] were prepared by purging the fluorocarbon, which exists in gaseous form at room temperature, through a needle inserted deep into the sealed serum bottle containing a known volume of polyethylene glycol 400[5]. The gas was allowed to escape the bottle through a 27-gauge needle inserted just below the surface of the stopper. After purging for 15 min, the needles were removed and the solution was set aside for at least 30 min. Then the rubber stopper was pierced with another 27-gauge needle to release any excessive pressure.

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

The amount dissolved was determined by the mass difference before and after purging. The solutions were then analyzed to confirm the concentration using a headspace equilibration method. This method and the detailed procedure for the preparation of sterile solution in polyethylene glycol 400 were described earlier (11).

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 a silicone tubing. The solution was infused over 3 min at a constant rate of 2.2 ml/min. At the end of the infusion, the cannula was flushed with 5 ml of heparinized sterile normal saline.

Blood samples (0.5–3.0 ml) were collected following the end of administration 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. A blank blood sample was obtained before the solution was administered to study the partition coefficient. The cannula through which the solution was administered was removed immediately following the administration. Every time a blood sample was withdrawn, the cannula was flushed with heparinized normal saline to avoid clotting.

During all of the studies, adequate room ventilation was maintained. Care was taken not to excite the animal since the cardiac output and breathing rate can affect the results significantly. No study was performed when the animal got highly excited during the cannulation process.

Analysis of Dichlorotetrafluoroethane in Blood—The blood samples collected were transferred to sealed, preweighed, heparinized serum bottles of various capacities and allowed to equilibrate with the gaseous phase of the bottle. Appropriate volumes of the gaseous phase were then analyzed using a gas chromatograph equipped with an electron-capture detector. The concentration in the blood sample was arrived at indirectly through the partition coefficient value; this value was determined using the headspace equilibration method described earlier[6] (12, 13).

The analyses were performed as soon as possible within 1 hr after sampling 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—An intravenous dosage form was chosen based on failures in the past to administer fluorocarbons quantitatively through the lungs (14, 15) and to avoid problems of biological availability in oral administration (16). The merits for the selection of polyethylene glycol 400 as the infusion solvent were discussed earlier (11).

Pharmacokinetic Model—For simplification in pharmacokinetic analyses of the blood level data, the zero time was assigned at the midpoint of infusion (17), *i.e.*, 1.5 min after the beginning of infusion. The blood level curves plotted on semilogarithmic paper were then subjected to the visual feathering method analysis (17, 18). A typical plot from one dog study is shown in Fig. 1. For clarity, the feathered data points in the faster biexponential phases are not shown in the figure. However, they all fell on or near the lines.

The triexponential blood level decay for all dogs studied indicates that the disposition kinetics for this propellant in dogs can be best described by a three-compartment open model (17–20) (Scheme I). The other two commonly used fluorocarbon propellants, trichloromonofluoromethane (11) and dichlorodifluoromethane (19), were also found to exhibit triexponential blood level decay patterns in dogs.

The two compartments besides the central compartment can be described as groups of tissues with distinct affinity and capacity (18) for dichlorotetrafluoroethane. The differences in affinity and capacity may be due to the solubility characteristics or to a specific interaction 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).

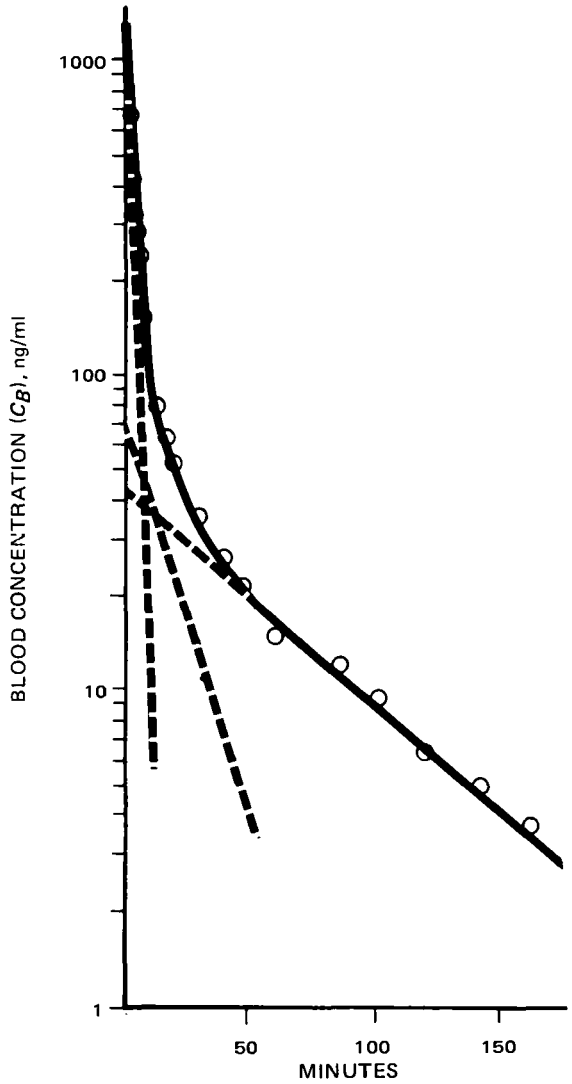


Figure 1—*Triexponential blood concentration decay following intravenous administration of 99 mg of dichlorotetrafluoroethane in 6.6 ml of polyethylene glycol 400 to a 19-kg dog:* $C_B = 1250e^{-0.375t} + 75e^{-0.0603t} + 42e^{-0.0161t}$.

The rate constants (Scheme I) were calculated using a digital computer[7], based on the differential equation solutions described by Rescigno and Segre (18).

The fraction of the dose remaining in various pharmacokinetic compartments can be described in terms of hybrid constants (18):

$$X_1/X_0 = C_1e^{-b_1t} + C_2e^{-b_2t} + C_3e^{-b_3t} \quad \text{(Eq. 1)}$$

$$X_2/X_0 = C_{12}e^{-b_1t} + C_{22}e^{-b_2t} + C_{32}e^{-b_3t} \quad \text{(Eq. 2)}$$

$$X_3/X_0 = C_{13}e^{-b_1t} + C_{23}e^{-b_2t} + C_{33}e^{-b_3t} \quad \text{(Eq. 3)}$$

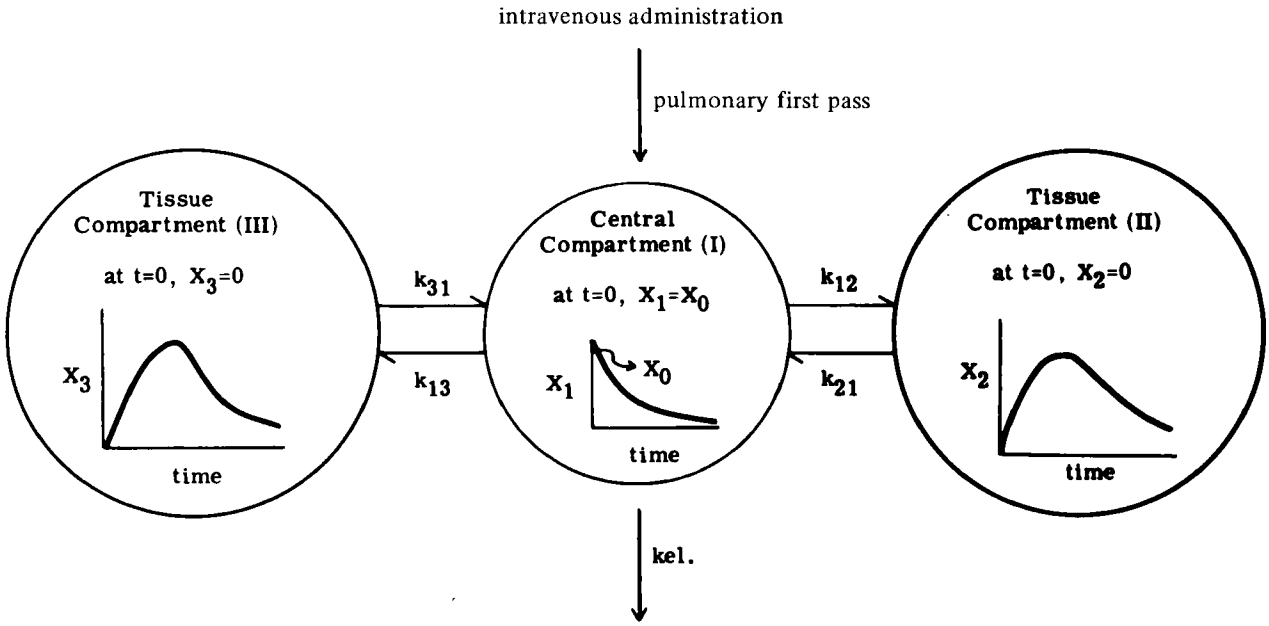
where X_1, X_2, and X_3 are the amounts of the fluorocarbon present in different compartments; X_0 is the available dose, which in the model proposed will be the dose remaining in the body after the first pass through lungs.

The pharmacokinetic data reported here are based on the single-dose study (Table I). The previous study of trichloromonofluoromethane in dogs showed dose-independent pharmacokinetic disposition kinetics over a wide dose range (11).

Volume of Distribution and Pulmonary Clearance—The dose of fluorocarbon administered intravenously is not the same as the dose made available to the body, since the intravenously administered dose first passes through the lungs, where a significant

[6] References 11 and 13 should be consulted for a detailed description of the procedure.

[7] IBM 370.



Scheme I—Three-compartment disposition model for dichlorotetrafluoroethane in dogs

fraction can be eliminated before it reaches the general body circulation. Therefore, the fraction eliminated in the first pass has to be accounted for in the calculation of the volume of distribution or pulmonary clearance.

The apparent volume of distribution, $(V_d)_{b_3}$, following pseudo-distribution equilibrium can be defined by the following equation (21), which takes into account the first-pass effect:

$$(V_d)_{b_3} = \frac{(PFR)(D^0)}{b_3[(AUC)(PFR + D^0)]} \qquad \text{(Eq. 4)}$$

where AUC is the area of the blood concentration–time curve from zero to infinity, b_3 is the first-order disposition constant in the terminal phase, D^0 is the dose administered, and PFR is the pulmonary blood flow rate (~cardiac output). Equation 4, however, assumes that the pulmonary clearance is the only route of elimination for the compound. The fluorocarbon studied in this investigation has been shown to be primarily eliminated intact from the lung (22). No volatile metabolite was detected in the GC assay in the present study. Therefore, Eq. 4 can be used as an approximation for the determination of the apparent volume of distribution and pulmonary clearance, PC:

$$PC = (V_d)_{b_3}(b_3) \qquad \text{(Eq. 5)}$$

The fraction eliminated in each pass through the lungs can be expressed as:

$$\text{fraction eliminated} = (1 - f) = \frac{PC}{PFR} \qquad \text{(Eq. 6)}$$

where f is the fraction of the administered dose retained in the body following the passage through the lung.

The volume of the central compartment, from where the elimination is assumed to take place, can be calculated easily since the clearance from the central compartment should equal the clearance from the body:

$$(V_d)_{b_3}(b_3) = V_c K_{el} \qquad \text{(Eq. 7)}$$

where K_{el} is the elimination rate constant from the central compartment.

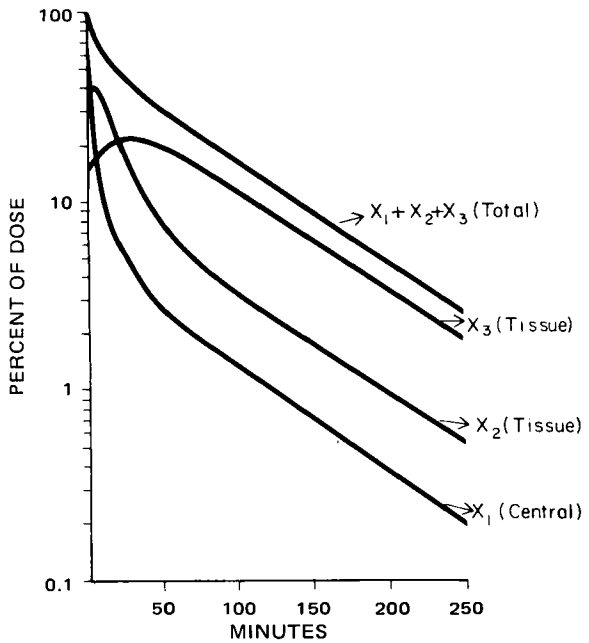


Figure 2—*Percent of available dose of dichlorotetrafluoroethane remaining in various body compartments based on a typical study (Dog 3287).*

Table II shows the values of the volumes of distribution, pulmonary clearance, and percent eliminated in each passage through the lungs. The average value of the volume of distribution, $(V_d)_{b_3}$, was approximately 10 times the average body weight (16.8–20 kg), indicating that this compound is indeed extensively distributed in the body tissues. Recent data (23, 24) have shown the possible contribution of tissue and plasma protein binding and partitioning in fat depots to the high volumes of distribution reported here.

The average pulmonary clearance volume of 2.53 liters/min was about 84% of the pulmonary blood flow rate (~3 liters) reported in the literature (25). This finding shows the magnitude of error that would have been involved if the correction for the first-pass effect had not been included.

Disposition Half-Lives—Table III shows the half-lives for the three disposition phases of dichlorotetrafluoroethane. The terminal disposition half-lives (~50 min) were much longer than previously reported or speculated (14, 26, 27) for two reasons. First, most studies were performed for very short periods within which the fluorocarbon was still in the distribution phase; and second, the analytical techniques were not sensitive enough to quantitate the low concentrations existing even after a short period following administration (Fig. 1).

The finding that dichlorotetrafluoroethane 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.

Table I—Pharmacokinetic Parameters of Dichlorotetrafluoroethane from Four Studies

Parameter[a]	Dog 3287	Dog 3288	Dog 3457	Dog 2976
b_1	0.737	0.375	0.577	0.591
b_2	0.0613	0.0603	0.107	0.0761
b_3	0.0120	0.0161	0.0157	0.0147
C_1	0.840	0.914	0.855	0.872
C_2	0.119	0.0548	0.137	0.101
C_3	0.0402	0.0307	0.00805	0.0263
C_{12}	−0.557	−0.265	−0.333	−0.409
C_{22}	0.454	0.224	0.325	0.363
C_{32}	0.103	0.0403	0.008	0.0454
C_{13}	−0.106	−0.185	−0.0687	−0.140
C_{23}	−0.271	−0.106	−0.0696	−0.172
C_{33}	0.377	0.292	0.138	0.313
K_{el}	0.156	0.190	0.305	0.217
K_{21}	0.162	0.0810	0.173	0.136
K_{31}	0.0217	0.0236	0.0183	0.0222
K_{13}	0.0900	0.0712	0.0449	0.0915
K_{12}	0.382	0.0850	0.158	0.213
D^0, mg[b]	297	99	281	160
X^0, mg[c]	38	17.7	51.6	22.3
$C_p{}^0$, mg/ml	2.26	1.37	6.43	1.88
AUC^∞, (mg/ml)/min[d]	14.5	7.18	21.1	8.63

[a] The units for all b and K values are minutes^{-1}. [b] Dose administered. [c] Available dose = fD^0, where f is the fraction retained in the first pass through lungs. [d] $AUC^\infty = C_p{}^0\left(\frac{C_1}{b_1} + \frac{C_2}{b_2} + \frac{C_3}{b_3}\right)$.

Table II—Volumes of Distribution and Clearance of Dichlorotetrafluoroethane

Dog	$(V_d)_{b_3}$, liters	V_c, liters	PC, liters/min	$(1-f)$, %
3287	217.10	16.81	2.62	87.20
3288	152.8	12.95	2.46	82.13
3457	155.5	8.02	2.45	81.64
2976	175.8	11.89	2.58	86.07
Mean ± *SEM*	175.31 ± 14.84	12.42 ± 1.81	1.53 ± 0.04	84.26 ± 1.39

The average half-life of the initial disposition phase was only 1.29 min. The actual value should be slightly smaller because this value was calculated after a 3-min infusion instead of an instantaneous administration.

Retention following Single Intravenous Administration—Following administration of dichlorotetrafluoroethane through intravenous infusion, the concentration in the blood dropped sharply. Such an observation was also reported for fluorocarbons following their inhalation (26, 27). However, such sharp declines in blood concentrations do not necessarily follow a parallel loss of the drug from the body (20). The amount of the drug remaining in the body as a function of time can also be calculated (20) using:

$$\text{fraction remaining in body} = \frac{(C_1/b_1)e^{-b_1t} + (C_2/b_2)e^{-b_2t} + (C_3/b_3)e^{-b_3t}}{(C_1/b_1) + (C_2/b_2) + (C_3/b_3)} \quad \text{(Eq. 8)}$$

Figure 2 shows a plot of amount of dichlorotetrafluoroethane remaining in the body and in various tissue compartments. For example, the amount of dichlorotetrafluoroethane in the central compartment dropped to about 5% of the available dose (dose after the first pulmonary pass) in 25 min, but about 45% of the available dose was still present in the body.

It is evident that a buildup of the fluorocarbon continues in the tissue compartments and that the maximum amount is reached at the steady state when the rate of change in the amount present in the tissue compartments equals zero; the amount present in the tissue compartments then declines. The ratio of the amount of the propellant in the tissue compartment 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 a pseudo-equilibration stage is reached; then the ratio of the amount in the tissue compartments to the amount in the

Table III—Disposition Half-Lives of Dichlorotetrafluoroethane

Disposition Phase	Half-Life (Mean + *SEM*), min
Initial	1.29[a] ± 0.19
Intermediate	9.60 ± 1.17
Final	50.8 ± 3.88

[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.

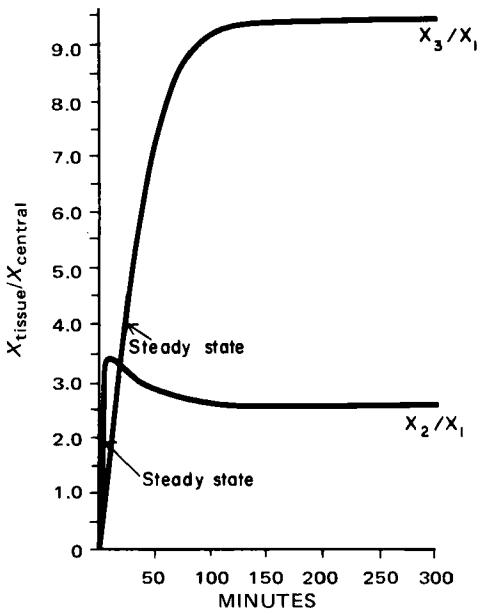


Figure 3—*Comparison of the relative amount of dichlorotetrafluoroethane remaining in the tissue and central compartments based on a typical study (Dog 3287).*

Table IV—Average Retention ± *SEM* of Dichlorotetrafluoroethane at 200 min following Pseudo-Distribution Equilibrium in Various Compartments

	$X_2 + X_3$ Compartments	X_1
Percent of available dose	1.74 ± 0.72	0.16 ± 0.01
Relative percent of total amount in the body	91.6 ± 37.9	8.4 ± 3.7

central compartment remains constant (28). To demonstrate this, a plot was made (Fig. 3) based on the data presented in Fig. 2. It took about 125 min to reach equilibration with the central compartment.

This finding is important since it shows that the claims made regarding the extremely fast elimination of the fluorocarbon from the body (14, 26, 27) may not be correct. Such a finding may have important implications in the evaluation of the toxicity of this compound.

In Compartment II (X_2), a peak was observed for the ratio of fluorocarbon in the tissue compartment over the amount in the central compartment. This kind of peak ratio was not observed for Compartment III (X_3) due to the slower accumulation in Compartment III. A constant ratio is obtained only when this compartment equilibrates with the central compartment.

The total amount of the available dose retained following pseudo-distribution equilibrium is an important parameter, especially when extrapolating the single-dose study to multiple-dose studies. Table IV shows the retention of dichlorotetrafluoroethane in the tissue and central compartments after pseudo-distribution 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 is retained in tissue compartments. Thus, 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.

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