Fluorocarbon Aerosol Propellants XI: Pharmacokinetics of Dichlorodifluoromethane in Dogs following Single and Multiple Dosing

SARFARAZ NIAZI and WIN L. CHIOU ˣ

Abstract □ A three-compartment open model was proposed for the disposition of dichlorodifluoromethane in dogs with average half-lives of 1.47, 7.95, and 58.5 min for the three disposition phases. This proposal is contrary to several studies that monitored blood levels for a shorter period. An analysis of the tissue compartment distribution following a single dose showed that about 1.5 hr was required to achieve pseudodistribution equilibration, following which more than 90% of the dose remaining in the body was retained in the tissue compartments. The pulmonary clearance and volumes of distribution were calculated considering the first-pass effect through the lungs. The volume of distribution after reaching pseudodistribution equilibrium was approximately 10 times the body weight in terms of the blood concentration, and about 68% of the propellant was cleared from the blood passing through the lungs in each cycle. Disposition of the propellant followed dose-independent kinetics after multiple dosing. No volatile metabolites were detected in the blood using GC.

Keyphrases □ Fluorocarbon aerosol propellants—dichlorodifluoromethane, single and multiple dosing, pharmacokinetic model proposed, dogs □ Aerosol propellants, fluorocarbon—dichlorodifluoromethane, single and multiple dosing, pharmacokinetic model proposed, dogs □ Dichlorodifluoromethane—single and multiple dosing, pharmacokinetic model proposed, dogs □ Pharmacokinetics—dichlorodifluoromethane, single and multiple dosing, three-compartment open model proposed, dogs □ Distribution—dichlorodifluoromethane, single and multiple dosing, pharmacokinetic model proposed, dogs

The toxicity aspects of fluorocarbon aerosol propellants reflected in their pharmacokinetics were reported recently (1–5). A similar approach is made here for the most commonly used fluorocarbon aerosol propellant, dichlorodifluoromethane, to show that this compound has a longer biological half-life than speculated and undergoes extensive distribution in body tissues. These findings may have bearing on the toxicity profile of this compound.

EXPERIMENTAL

Dichlorodifluoromethane[1] was administered intravenously in polyethylene glycol 400 solution to four male mongrel, unanesthetized, conditioned dogs, 16.8–20 kg. The doses of dichlorodifluoromethane administered ranged from 71 to 135 mg. Procedural details for the preparation of the dosage forms, animal preparation, single- and multiple-dose administrations, blood samples, and analysis of fluorocarbon in the blood samples were reported earlier (3, 4). The blood samples were analyzed as soon as possible to avoid any loss during storage (6). A duplicate study was performed on Dog 2976.

RESULTS AND DISCUSSION

Dosage Form and Route of Administration—The intravenous dosage form was selected to avoid the problems of biological availability in inhalation and oral administration (7–10). This mode of administration would be equivalent to inhalation (3, 4) if one considers that the intravenously administered fluorocarbon first passes through the lungs before being monitored in the blood. The fluorocarbon concentration profile in blood would, therefore, appear as if the fluorocarbon had been administered *via* inhalation, the customary route of its uptake.

Pharmacokinetic Model—In all instances, the blood concentration–time curves were characterized by three exponential equations, which can be represented as a three-compartment open model for the disposition of dichlorodifluoromethane (Fig. 1). The two compartments besides the central compartment can be described as the group of tissues with distinct affinity and capacity for dichlorodifluoromethane. The difference in the affinity may be due to solubility characteristics or to specific interactions such as protein binding or other complexations (11–15). A compartment can also be distinguished based on the blood supply to the tissues such as the vessel-rich group and the vessel-poor group (14, 16).

For further analysis of this model (Scheme I) to calculate various rate constants, a digital computer program was generated based on reported differential equation solutions (17).

The fraction of dose remaining in various pharmacokinetic compartments can be described in terms of hybrid constants (17):

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

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

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

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

The values of these pharmacokinetic parameters calculated for dichlorodifluoromethane are summarized in Table I.

Recently, Adir *et al.* (10) also proposed a pharmacokinetic model for this propellant, suggesting a two-compartment body distribution in dogs.

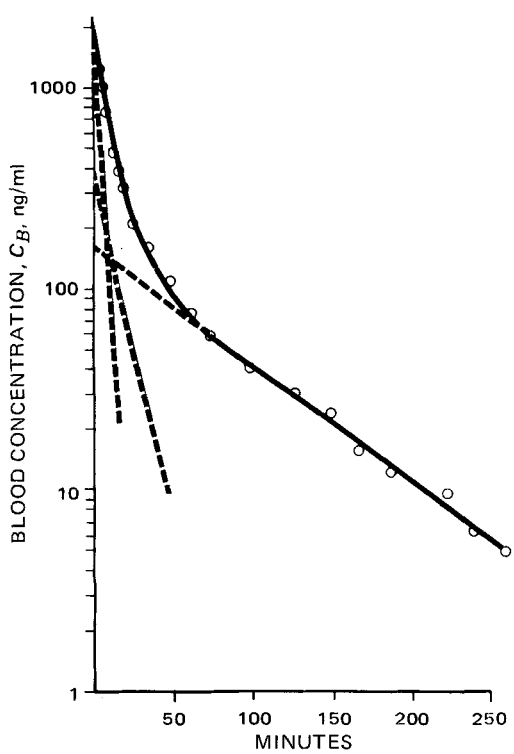
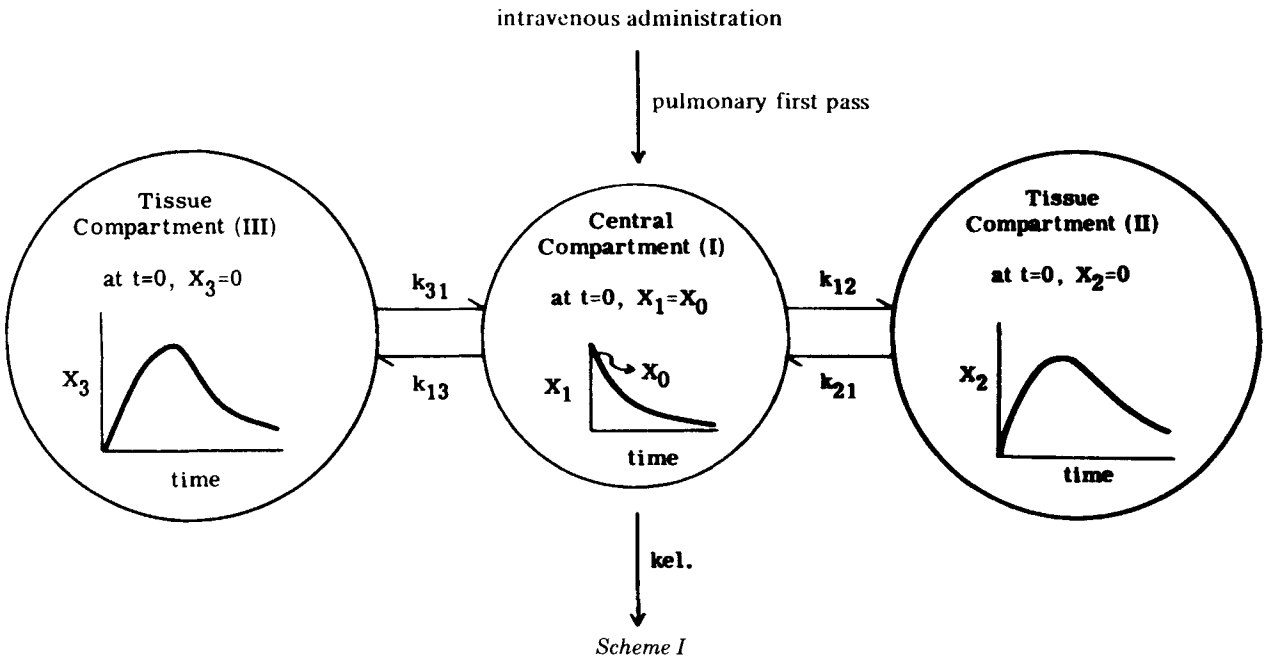


Figure 1—*Triexponential blood concentration decay following intravenous administration of 105.0 mg of dichlorodifluoromethane in 6.6 ml of polyethylene glycol 400 to a 19-kg dog:* $C_B = 1200e^{-0.227t} + 375e^{-0.079t} + 164e^{-0.016t}$

[1] E. I. du Pont De Nemours & Co., Wilmington, Del.



Scheme I

However, their study (10) monitored the blood levels following inhalation for only about 20–30 min, which does not allow for the elucidation of equilibration with the deeper tissue compartment. A comparison of initial and intermediate rate constants (b_1 and b_2 in Table I) shows good agreement with the values reported recently (10). For example, our average value of 0.60 min^{-1} for b_1 compared with 0.64 min^{-1} (10) and the value of 0.09 min^{-1} for b_2 compared with 0.07 min^{-1} (10). However, the actual values of these rate constants in the study of Adir *et al.* (10) will be higher if the deeper tissue compartment is "peeled off" from the observed blood concentrations.

Volume of Distribution and Pulmonary Clearance—In the present study, the dose administered is not the same as the dose made available to the body, since the total dose passes through the lungs where a significant fraction can be eliminated before it is distributed to the body. Therefore, an equation correcting for this first-pass effect was used to calculate the apparent volume of distribution following pseudodistribution equilibrium (3, 4, 18):

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

where *PFR* is the pulmonary flow rate (~cardiac output), *AUC* is the total area under the blood concentration–time curve (extrapolated to time infinity), and D^0 is the dose administered. Equation 4, however, assumes that the pulmonary clearance is the only route of elimination for the compound. In the present study, no volatile metabolites could be detected from the blood sample by the GC analysis. Since the fluorocarbon studied in this investigation has been shown to be primarily eliminated intact from the lungs (19), Eq. 4 can be used as an approximation for the determination of the 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, $(1 - f)$, can be calculated as:

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

Table II shows the values of the volume of distribution following pseudodistribution equilibrium to be almost 10 times the body weight. This finding indicates an extensive distribution of dichlorodifluoromethane to the body tissues. Recent findings on the binding of the propellant to human and bovine albumin (11) suggest that the propellant may be bound also to other tissue components. The possible high solubility in the fat tissue due to its lipophilic physical property also may contribute to the high apparent volume of distribution, as was found for trichloromonofluoromethane in dogs (3, 15) and chloroform in humans (20).

Table I—Pharmacokinetic Parameters of Dichlorodifluoromethane from Five Studies

Parameter[a]	Dog 3287	Dog 3288	Dog 3457	Dog 2976a	Dog 2976b
b_1	0.99	0.495	0.840	0.227	0.495
b_2	0.139	0.0894	0.0729	0.079	0.0792
b_3	0.0133	0.0103	0.0133	0.0157	0.009
C_1	0.967	0.920	0.843	0.690	0.904
C_2	0.0281	0.0763	0.134	0.216	0.0914
C_3	0.00445	0.00340	0.0231	0.0943	0.00452
C_{12}	−0.149	−0.242	−0.537	−0.261	−0.300
C_{22}	0.146	0.239	0.482	0.223	0.296
C_{32}	0.00379	0.00302	0.0548	0.0376	0.00509
C_{13}	−0.172	−0.0676	−0.0870	−0.170	−0.0772
C_{23}	−0.0400	−0.0348	−0.214	−0.208	−0.0549
C_{33}	0.212	0.102	0.301	0.378	0.132
K_{el}	0.661	0.328	0.218	0.0851	0.287
K_{21}	0.163	0.121	0.189	0.119	0.118
K_{31}	0.017	0.011	0.0198	0.0280	0.0104
K_{13}	0.173	0.0355	0.084	0.0492	0.0414
K_{12}	0.128	0.0984	0.415	0.0410	0.125
D^0b, mg	71	178	103	105	135
AUC^∞, μg/ml × min	15.6	16.5	16.0	20.4	18.10

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

Table II—Volumes of Distribution and Clearance of Dichlorodifluoromethane

Dog	$(V_d)_{b_3}$, liters	V_c, liters	PC, liters/min	$(1-f)$, %
3287	135.5	2.73	1.81	60.21
3288	228.5	7.14	2.35	78.20
3457	153.4	9.37	2.04	68.17
2976a	120.3	22.25	1.89	63.14
2976b	237.7	7.45	2.14	71.31
Mean ± *SEM*	175.1 ± 24.3	9.79 ± 3.30	2.05 ± 0.09	68.21 ± 3.15

The average pulmonary clearance values show a clearance of 2.05 liters/min, corresponding to 68% clearance in each passage through the lungs. An error of this magnitude would have been made in the calculation of the volume of distribution had the correction due to the first-pass pulmonary effect not been made, as explained earlier.

The volume of the central compartment, from which the actual elimination takes place, can be calculated easily, since the clearance from the central compartment should be equal to 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. Table II also shows the values for the volumes of the central compartment. Smaller volumes as compared to the total volumes of distribution indicate the retention of a smaller fraction in the central compartment as compared to tissue compartments after the pseudodistribution phase.

Disposition Half-Lives—Table III shows the half-lives for three disposition phases of dichlorodifluoromethane. The terminal disposition half-lives reported here are much longer than those previously reported or speculated (7, 8, 10, 21–23).

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

Retention following Single Intravenous Administration—Following administration of dichlorodifluoromethane through intravenous infusion, the concentration in the blood dropped sharply. Such an observation also was reported for fluorocarbons following their inhalation (10, 21–23). However, such sharp declines in blood concentrations do not necessarily follow a parallel loss of drug from the body (24). The amount of drug remaining in the body as a function of time can also be calculated using (24):

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

Figure 2 shows a plot of the amount of dichlorodifluoromethane remaining in the body and in various tissue compartments, as described previously. For example, the amount of dichlorodifluoromethane in the central compartment dropped to about 12% of the absorbed dose (dose after the pulmonary first pass) in 25 min, but about 45% of the absorbed dose was still present in the body.

Although the steady state was reached rather quickly, as indicated by arrows in Fig. 3, the ratio of the amount in tissue compartments over that in the central compartment continues increasing until attainment of the pseudodistribution equilibrium, which would take about 90 min. This finding is of importance because it was quite contrary to previous claims of fast elimination of fluorocarbons from the body (8, 21–23).

The total amount of the absorbed dose retained following pseudodistribution equilibrium is an important parameter, especially for extrapolating the single-dose study to multiple-dose studies. Table IV shows the retention of dichlorodifluoromethane in the tissue and central compartments after pseudodistribution equilibrium has been reached. The fraction of the fluorocarbon retained in the tissue compartments compared to that in the central compartment shows that more than 90% of the total amount present in the body is retained in tissue compartments.

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 the one with Dog 2976a, was used to generate[2] multiple-dose disposition curves. These curves were then superimposed on 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 decayed independently of the amount present in the body. In other words, dose-independent kinetics were operative for the disposition of this compound in the dose range studied.

Table III—Disposition Half-Lives of Dichlorodifluoromethane

Disposition Phase	Half-Life (Mean ± *SEM*), min (n = 5)
Initial	1.47 ± 0.42[a]
Intermediate	7.95 ± 0.79
Final	58.50 ± 5.99

[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.

Table IV—Average Retention ± *SEM* of Dichlorodifluoromethane at 200 min following Pseudodistribution Equilibrium in Various Compartments

Retention	$X_2 + X_3$ Compartments	X_1 Compartment
Percent of absorbed dose	1.88 ± 0.22	0.14 ± 0.07
Relative percent of total amount in body	93.0 ± 10.9	7.0 ± 3.5

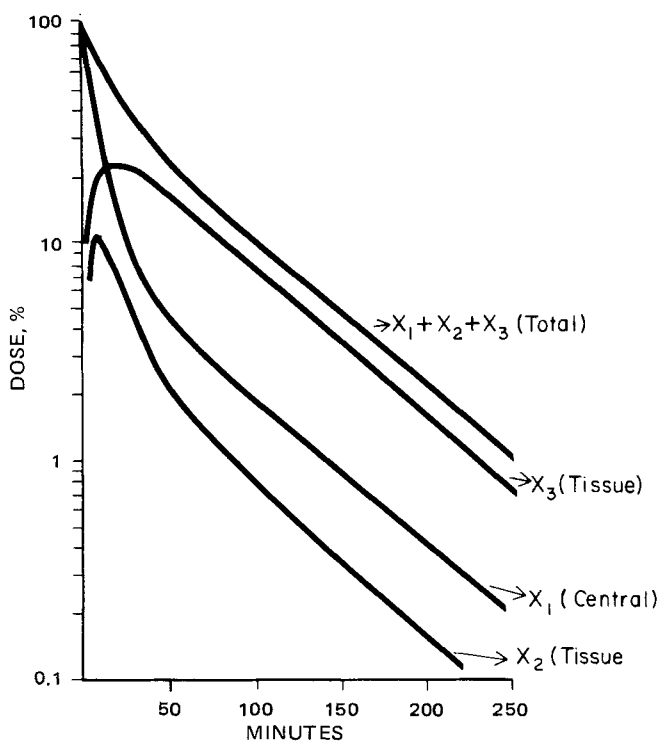


Figure 2—*Percent of absorbed dichlorodifluoromethane remaining in various body compartments based on a typical study (Dog 2976a).*

[2] IBM 370 computer at the University of Illinois Medical Center.

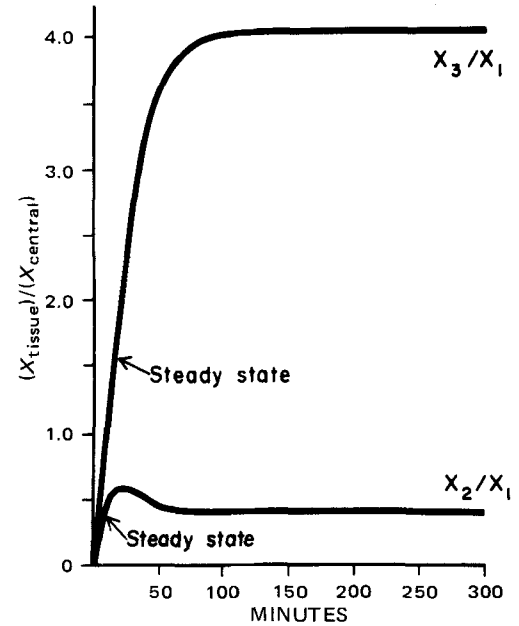


Figure 3—*Comparison of the relative amount of dichlorodifluoromethane remaining in tissue and central compartments based on a typical study (Dog 2976a).*

This approach allows the assessment of the accumulation of dichlorodifluoromethane in the tissue compartments during multiple exposures in the frequent use of commercial products containing fluorocarbon propellants. Figure 5 shows the calculated amount of dichlorodifluoromethane distributed to various tissue compartments following six doses. The magnitude of the problem of accumulation during multiple exposures to dichlorodifluoromethane 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 (Fig. 5).

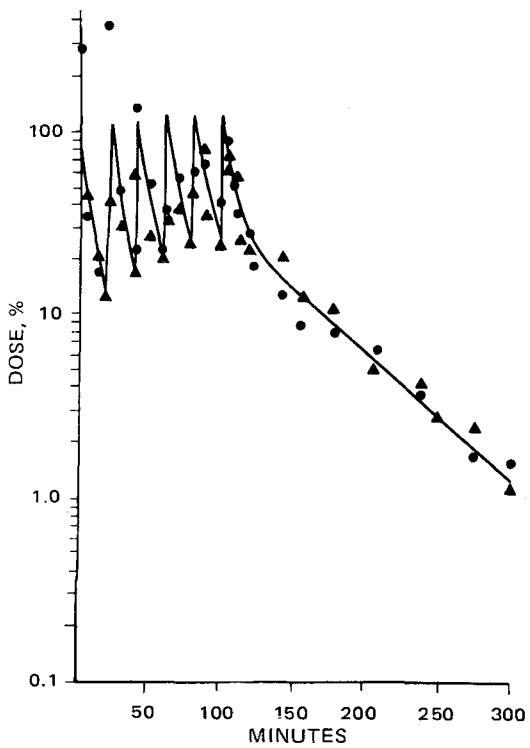


Figure 4—*Percent of absorbed dose remaining in the central compartment following six intravenous administrations of 74 (●) or 89 (▲) mg of dichlorodifluoromethane every 20 min. The solid curve represents the theoretical values based on a typical set of pharmacokinetic parameters obtained from a single-dose study (Dog 2976a).*

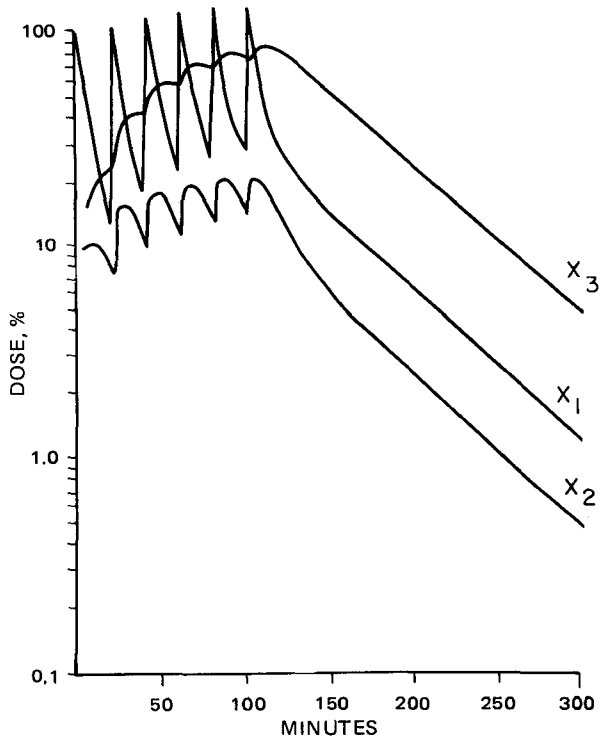


Figure 5—*Computer-generated curves for the distribution of dichlorodifluoromethane in various pharmacokinetic compartments following instantaneous absorption of six doses of 100 units each every 20 min. The pharmacokinetic parameters were obtained from a typical single-dose study (Dog 2976a).*

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 dichlorodifluoromethane. Sudden deaths reported following inhalation of aerosol packages can be attributed, at least in part, to the reaching of threshold toxic limits, although no such direct and reliable data have ever been reported. Second, the accumulation following multiple exposures can result in much higher deep tissue compartment levels than would be apparent from blood levels. Hence, 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 warn against misleading toxicological interpretations that result from the application of erroneous pharmacokinetic principles.

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Distribution and Pharmacokinetics of Triamterene in Rats

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Abstract □ The tissue distribution of ^{14}C-triamterene was examined in the rat. After intravenous administration of ^{14}C-triamterene, high concentration ratios between tissues and blood were found in most tissues except the brain, fat, and testes. The maximal concentration of the drug was in the kidneys, liver, heart, lungs, and skeletal muscle within the first 20 min, when the maximal natriuresis was observed. No metabolite of triamterene was detected in these tissues. The pharmacokinetics of ^{14}C-triamterene also were investigated. The volume of distribution of the drug was greater in the central compartment (60% of the dose) than in the peripheral compartment (40%). The binding of the drug to skeletal muscle is responsible for the fraction of the dose in the peripheral compartment. Rate constants indicate that slow elimination of triamterene is related to its binding to tissue in the central compartment.

Keyphrases □ Triamterene—distribution and pharmacokinetics, radiochemical analysis, rats □ Drug distribution—triamterene, radiochemical analysis, rats □ Pharmacokinetics—triamterene, radiochemical analysis, rats □ Radiochemistry—analysis, triamterene distribution, rats □ Diuretic agents—triamterene, distribution and pharmacokinetics, radiochemical analysis, rats

Triamterene, in addition to its renal effects, significantly decreases the sodium content of cardiac and skeletal muscle without impairing the potassium content in the rat (1). Although an unusual tissue distribution of triamterene was reported in guinea pigs and baboons (2), its distribution in the rat has not been studied. Previous studies (3, 4) indicated that unchanged triamterene itself, rather than its metabolites, accounts for the observed changes of renal clearance of electrolytes.

It is not known whether the metabolism of triamterene in cardiac and skeletal muscle is similar to that in the kidneys in the rat. The contribution of the drug distributed in the tissue and of its metabolites to the total elimination of triamterene by humans and animals also is not known. The purpose of this study was to determine the tissue distribution and pharmacokinetics of triamterene in the rat to obtain a better understanding of the rate of distribution and elimination of triamterene and its extrarenal action.

EXPERIMENTAL

Animals and Materials—Male Sprague–Dawley rats[1], 200–250 g, were used. ^{14}C-Triamterene[2] (0.92 mCi/mmole) was used as supplied. It was found to be chemically pure by descending paper chromatography in two solvent systems, 1-butanol–acetic acid–water (4:1:1) and 2-propanol–water–ammonia (140:50:10), according to literature methods (4–6). A 4 × 4 countercurrent distribution of ^{14}C-triamterene between ethyl acetate (14 ml) and 0.1 *M* borate buffer at pH 10.00 (7 ml) showed that it behaved like a single compound (6–8).

Other commercially available chemical reagents were used.

Tissue Distribution—Rats were prepared for infusion as described previously (9), and ^{14}C-triamterene[3] (1, 2, or 4 mg/kg; 3.8 μCi/mg) was administered by intravenous infusion for 8 min at 0.5 ml/min/kg. Samples of blood and urine were collected 40 min after the beginning of the infusion, and the rat was then immediately decapitated. Submaxillary and thyroid glands, thymus, aorta, heart, lungs, liver, pancreas, spleen, adrenals, kidneys, ileum, biceps femoris, epididymal fat pads, testes, and brain were dissected, blotted, and weighed. The distribution of ^{14}C-triamterene and its metabolites was determined at 40 min, when the maximal potassium-sparing effect of triamterene was observed (3).

In a second series of experiments, a constant dose of ^{14}C-triamterene (2 mg/kg, 3.8 μCi/mg) was administered by infusion; the rats were sacrificed at intervals of 10, 20, 40, or 60 min thereafter. Plasma, liver, lung, heart, kidney, skeletal muscle (biceps femoris), and urine samples were collected.

The total carbon-14 in the tissues samples was measured[4]. About 200 mg of tissue was placed into a 20-ml glass counting vial. A 2-ml aliquot of liquid scintillation solute[5] was added, and the sample was shaken[6] at 50° until solubilized. To increase the counting efficiency of some heavily colored tissues (blood, liver, lungs, and spleen), 0.6 ml of benzoyl peroxide solution was added to the solubilized sample, and the mixture was shaken for an additional 0.5 hr to obtain a colorless solution.

To the solubilized tissues, 18 ml of counting solution[7] was added. The samples were cooled to 4° for 24 hr prior to counting. All samples were counted twice for 10 min. All data were converted to disintegrations per

[1] Holtzman, Inc., Madison, Wis.
[2] Smith Kline and French Laboratories, Inc.
[3] Triamterene was solublized in Solution A, containing inulin (0.15%), aminohippuric acid (0.2%), and ketamine (8 mg/kg/ml), with the aid of a few drops of 8.5% lactic acid and warming. The total volume administered was 4 ml/kg.
[4] Packard liquid scintillation counter.
[5] Soluene, Packard Instrument Co.
[6] Dubnoff metallic shaker.
[7] Phase Combing System, Amersham/Searle Corp.