formed and rapidly eliminated. The virtually instantaneous biotransformation of flunitrazepam to its *N*-desmethyl metabolite, the major blood component seen following both intravenous and oral administrations of the drug, suggests a first-pass effect. The apparent half-life of elimination of the *N*-desmethyl metabolite following intravenous administration of flunitrazepam was 4.7 and 6 hr in the two dogs studied. No measurable levels of either flunitrazepam or *N*-desmethyl-flunitrazepam were seen in the urine, indicating extensive and complete biotransformation and possible alternative routes of excretion.

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[x] To whom inquiries should be directed.

Partition Coefficients of Fluorocarbon Aerosol Propellants in Water, Normal Saline, Cyclohexane, Chloroform, Human Plasma, and Human Blood

SARFARAZ NIAZI and WIN L. CHIOU[x]

Abstract □ Partition coefficients of the three most commonly used fluorocarbon propellants (trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane) in water, normal saline, cyclohexane, chloroform, human plasma, and human blood were studied over a wide range of propellant concentrations. They were all found to be independent of concentration. No salting-out effect was seen in normal saline. The partition coefficients in plasma and blood were much greater than in normal saline. The implications of the results of this study on absorption, distribution, elimination, and assay of these propellants are discussed.

Keyphrases □ Fluorocarbon aerosol propellants—partition coefficients in six solvents □ Aerosol propellants, fluorocarbon—partition coefficients in six solvents □ Partition coefficients—determined for fluorocarbon aerosol propellants, six solvents □ Dichlorodifluoromethane—partition coefficient in six solvents □ Dichlorotetrafluoroethane—partition coefficient in six solvents □ Trichloromonofluoromethane—partition coefficient in six solvents

Solubilities of many volatile or gaseous anesthetic compounds in a liquid medium such as blood and olive oil have been often defined as being equivalent to their partition coefficients between the liquid phase and gaseous phase at an equilibrium state. The importance of this parameter to the absorption, distribution, elimination, and pharmacological activities of many compounds in humans and animals has been excellently reviewed (1). Volatile fluorocarbons such as trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane have been widely used as propellants in aerosol products for cosmetic, pharmaceutical, household, and other purposes. The toxicity of these fluorocarbon propellants has been a subject of intensive research and controversy in recent years, as was clearly illustrated in an editorial (2) and a rebuttal (3) to that editorial published recently.

After inhalation in human subjects, Paterson *et al.* (4) were unable to quantify dichlorodifluoromethane and dichlorotetrafluoroethane in blood. The fast elimination from the body and loss to the air from blood samples due to their high volatility or low solubility in blood were implicated as possible causes. More recently, Morgan *et al.* (5) correlated the observed slower tracheal absorption rates in humans of dichlorodifluoromethane and dichlorotetrafluoroethane with their lower solubilities in olive oil. Although they also reported the solubilities of four fluorocarbon propellants at one concentration for

each propellant in human serum, the solubilities in water, normal saline, human plasma, and human blood were not studied by them. It was felt that these data were also important for the better understanding of their pharmacokinetic properties in humans and animals. Therefore, this investigation was undertaken to study their solubilities or partition coefficients over a wide range of concentrations in water, normal saline, cyclohexane, chloroform, human plasma, and human blood.

The inclusion of cyclohexane in the study was prompted by a report (6) that this organic solvent was used to represent the lipoid phase in a partition coefficient study and the results correlated well with the biological activities of many drugs. It was also used as a solvent in this investigation for the fluorocarbon propellants to establish the standard curves for GC analyses. Only the three most widely used fluorocarbon propellants (trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane) were studied.

EXPERIMENTAL

Materials—The three high purity fluorocarbons[1], normal saline USP[2], cyclohexane[3] (99 mole %), and chloroform[4] were obtained from commercial sources. The human blood[5] used was pooled from blood samples of four healthy subjects and contained 12.5% (v/v) of anticoagulant citrate and dextrose solution USP. The plasma was obtained by centrifugation of the blood.

The standard solutions of the three fluorocarbon propellants used for GC analyses were prepared by the following method. Four milliliters of cyclohexane was transferred to a 5-ml capacity serum bottle[6] which was then quickly sealed with a flange-type rubber stopper[6,7] and an aluminum seal using a manual crimper. The bottle was weighed and care was taken to avoid wetting of the rubber stopper by cyclohexane. The trichloromonofluoromethane solution was prepared by drawing some refrigerated liquid trichloromonofluoromethane into a precooled (5°) glass syringe through a 22-gauge hypodermic needle, and a few drops were injected into the sealed bottle containing cyclohexane. Then the bottle was reweighed. A difference in weight, ranging from 20 to 80 mg, gave the amount of the propellant dissolved in cyclohexane.

The solutions of dichlorodifluoromethane and dichlorotetrafluoroethane were prepared by purging them in the gaseous state into the sealed serum bottle containing cyclohexane. This was accomplished by the following procedure. The pressure tank containing the liquid propellant was connected to a 22-gauge hypodermic needle by a rubber tubing through which the flow rate could be controlled by adjusting a clamp. Very low flow rates were used to avoid any pressure build-up inside the bottle. After purging the surface of cyclohexane with the gas for about 5 sec, the needle was withdrawn and another 22-gauge hypodermic needle was inserted just enough to pierce through the stopper to release any pressure inside the bottle. The bottle was then shaken gently and reweighed. These solutions also contained about 20–80 mg of the propellant. Every time a needle was inserted in a bottle, care was observed to avoid contacting cyclohexane to prevent its possible loss through the needle. Further dilutions were made with cyclohexane by volume. It is believed that this method of preparation of standard solutions can be more easily and accurately carried out than the method used by Shargel and Koss (7). Their method required handling at very low temperature and data on the densities of some propellants.

The concentrated solutions of dichlorodifluoromethane or dichlorotetrafluoroethane in normal saline were prepared by purging the gas slowly from the pressure tank, into a 50-ml capacity serum bottle filled with normal saline, through an 18-gauge hypodermic needle inserted well below the surface of the solution. The gas was allowed to escape the bottle during the purging through another hypodermic needle inserted just enough to pierce the rubber stopper. The concentrated solutions of trichloromonofluoromethane in normal saline were prepared by adding 1 ml of the liquid propellant to a serum bottle (15–50-ml capacity) filled with normal saline; the bottle was then sealed and shaken for 15 min and set aside for about 30 min to achieve a clear phase separation. The aqueous layer was then removed using a 50-ml glass syringe and injected into an empty serum bottle which was quickly sealed. The stock solution so prepared was kept filled to the top of the bottle to avoid any excessive loss due to partitioning into the gaseous phase. Proper dilutions of the three propellant stock solutions with normal saline were then made to give a wide range of concentrations for each propellant: about 40–1000 μg/ml for trichloromonofluoromethane, 20–200 μg/ml for dichlorodifluoromethane, and 50–100 μg/ml for dichlorotetrafluoroethane.

The exact concentrations of the propellants in normal saline were determined by injecting[8] 10 μl of the solution into an empty, sealed serum bottle of 15–50-ml capacity and analyzing the gaseous phase for the propellant concentration after shaking[9] the bottle for 5 min. The peak heights of the symmetrical gas chromatograms were then converted to amounts of the propellants injected using the standard curves prepared from the standard solutions in cyclohexane. The concentration of the original propellant concentration in normal saline was calculated by the following equation:

$$\text{concentration in normal saline} = \frac{\text{concentration in gaseous phase} \times \text{internal volume of bottle}}{\text{volume of propellant solution in normal saline added}} \quad \text{(Eq. 1)}$$

This method of calculation was accurate to at least 99.9%, because the volume of the normal saline propellant solution in the bottle was negligible compared to that of the gaseous phase and the solubilities of the propellants studied in normal saline were low. The internal volume of the serum bottle used in this study had been determined previously by the gravimetric method (weighing the sealed bottle before and after it was filled with water and then dividing the difference in weight by the water density at room temperature).

Solubility Determination—Samples for solubility studies were prepared by injecting 10 μl of concentrated propellant solutions in normal saline into 5 or 10 ml of distilled water, normal saline, plasma, or blood contained in the sealed serum bottle with a capacity of 15, 25, or 50 ml. The preparation of cyclohexane samples was the same as that described previously. The propellant solutions in chloroform were prepared by injecting 10 μl of known concentrated propellant solutions in cyclohexane into the sealed serum bottle containing chloroform.

The propellant samples were shaken[9] for 5 min. They were then placed in a constant-temperature water bath[10] maintained at 25° or, in some instances, at 37° and shaken at 100 rpm for 30 min. The concentrations of the propellants in both the liquid phase and head space were analyzed directly by the GC method. However, only the head space was analyzed in the studies of chloroform and plasma or blood in the case of dichlorotetrafluoroethane. To obtain maximum accuracy (using a larger volume for injection) and to avoid the GC detector response beyond the linear range, the high concentration gaseous samples were further diluted with air in 15–50-ml, sealed, empty bottles before analysis. Shaking[9] for 5 min was enough to reach an equilibrium state for the partitioning of the propellants between liquid and gaseous

[1] Supplied by E. I. duPont de Nemours & Co., Wilmington, Del.
[2] McGaw Laboratories, Glendale, Calif.
[3] Fisher Scientific Co., Fairlawn, N.J.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Obtained from the University of Illinois blood bank.
[6] Wheaton Scientific, Millville, N.J.
[7] This stopper was preferred because it had a lacquer coating which could reduce or prevent sorption of propellants onto the rubber stopper. It was obtained from West Co., Phoenixville, Pa.
[8] Hamilton syringe 700, Supelco, Inc., Bellefonte, Pa.
[9] Vortex, Fisher Scientific Co., Springfield, Mass.
[10] Eberbach Corp., Ann Arbor, Mich.

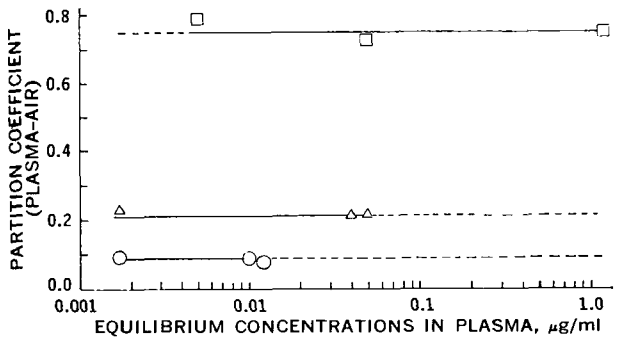

Figure 1—*Partition coefficients of fluorocarbon aerosol propellants between plasma and air as a function of concentration. Key:* □, *trichloromonofluoromethane;* ○, *dichlorodifluoromethane; and* △, *dichlorotetrafluoroethane.*

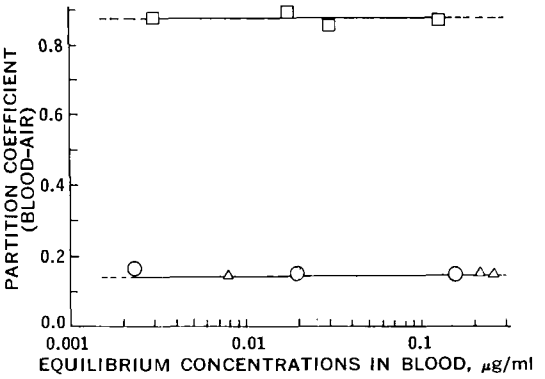

Figure 2—*Partition coefficients of fluorocarbon aerosol propellants between blood and air as a function of concentration. Key:* □, *trichloromonofluoromethane;* ○, *dichlorodifluoromethane; and* △, *dichlorotetrafluoroethane.*

phases. The concentrations of the propellants in the head space were essentially constant even after 2 hr of shaking.

The solubility, S, was calculated directly by dividing the concentration of the propellant in the liquid phase by that in the gaseous phase. It was also estimated indirectly by the following head-space method. After injecting the amount of the propellant contained in the 10 μl of normal saline, A_t, to the volume of the liquid phase, V_l, in the sealed serum bottle with a total internal volume of V_t, the following relationship based on material balance was obtained:

$$A_t = C_h(V_t - V_l) + C_lV_l \qquad \text{(Eq. 2)}$$

where C_h and C_l are the concentrations in the head space and liquid phase, respectively, at the equilibrium state. After rearranging Eq. 2:

$$S = \frac{C_l}{C_h} = \frac{A_t - C_h(V_t - V_l)}{C_hV_l} \qquad \text{(Eq. 3)}$$

Equation 3 enables one to estimate S by merely determining C_h since A_t, V_t, and V_l can be easily predetermined.

GC Analysis—A dual-column gas chromatograph[11] equipped with a tritium foil electron-capture detector was used. A 1.82-m (6-ft) U-shaped glass column (4 mm i.d.) packed with Porapak Q[12] (80–100 mesh) was used. The column temperature was 170°, and the injection port and the detector were maintained at 175°. The carrier gas (nitrogen) was used at a flow rate of 85 ml/min. For analysis of liquid samples, 1–10 μl was injected onto the column using a 10-μl syringe. For analysis of the head-space samples, 5–100 μl of the gaseous phase was injected onto the column using 50- or 100-μl gastight syringes[13]. All GC analyses were performed at least in duplicate, and reproducible results were obtained throughout the study. The retention times relative to the air peak were 0.9 min for dichlorodifluoromethane, 1.75 min for dichlorotetrafluoroethane, 3.25 min for trichloromonofluoromethane, 11 min for cyclohexane, and 8.0 min for chloroform.

RESULTS AND DISCUSSION

The average solubility data of the three fluorocarbon propellants determined at different concentrations in various media are summarized in Table I. No difference in solubility data could be found when determined either at 25 or 37°. In the past (1, 5, 8, 9), the solubilities of many anesthetic gases were often determined only at one concentration, assuming that they were independent of the concentration of the gases. Such an assumption may be wrong, for example, if the percentage of plasma protein binding of the anesthetic varies with its concentration in the plasma since the molecule in the head space is only thermodynamically in equilibrium with the unbound molecule and not with the bound molecule. The concentration-dependent protein binding of nonvolatile drugs is well known. Plots of solubilities of the propellants in human plasma and blood *versus* their equilibrium concentrations in plasma and blood are shown in Figs. 1 and 2. Within experimental errors, these results indicate that the solubilities or partition coefficients in plasma and blood were independent of concentration in the wide concentration range studied. Similar results were obtained in studies using water, normal saline, cyclohexane, and chloroform.

Identical results were obtained from the two methods of solubility determination for trichloromonofluoromethane and dichlorodifluoromethane. These findings were valuable because they showed that the simple indirect head-space method could be employed when the direct injection of the liquid phase such as plasma or blood onto the GC column was not desirable (7). Although the head-space method was used recently to study the solubilities of several volatile anesthetics in human and dog blood (9), the equilibration flask and tube used were more complicated than the serum bottle used in this study. Cowles *et al.* (9) used 50 ml of blood in each study, while 1–2 ml of the sample was enough in this study when using a 5-ml capacity bottle (currently used in this laboratory to study the solubilities of propellants in dog plasma and blood). The equilibration time used in their method was also much longer. Furthermore, the present method permits duplicate assay from the same bottle, and this is not easily feasible with their equilibration tube because some gas sample may be lost through the punctured hole of the parafilm.

The solubilities, expressed in terms of weight percent, of many fluorocarbon propellants in water at 1 atm and 25° were reported previously (10). Their solubility values as defined in this study can be theoretically calculated using the law of Gay-Lussac (11). The theoretical values for the three propellants studied are also shown in Table I. It is obvious that both the experimental and theoretical data are in good agreement. No salting-out effect was seen in normal saline, because the solubilities determined in water and normal saline were identical. This seems to indicate that hydration was not an important factor for the solution of these small and hydrophobic molecules.

The solubilities of the three propellants in plasma were all higher than those in normal saline. This was especially significant for trichloromonofluoromethane (3.5 times higher) and dichlorotetrafluoroethane (11 times higher). The enhancement of solubility was probably due to the binding of the propellants with proteins in the plasma. Further work is required to establish the mechanism for such a marked solubilization effect. The solubilities of trichloromonofluoromethane and dichlorodifluoromethane were higher in blood than in plasma, whereas the solubility of dichlorotetrafluoroethane was higher in plasma than in blood. These findings were similar to those of a previous report (9) which showed that the solubilities of ethylene and cyclopropane increased with an increase in hematocrit while halothane and ether showed an opposite effect.

The previously reported (5) solubility values for the three propellants in human serum are shown in Table I. Compared with the solubility data in plasma, it seems difficult to explain why

[11] Packard model 824, Packard Instrument Co., Downers Grove, Ill.
[12] Water Associates, Framingham, Mass.
[13] Hamilton syringe 7000, Supelco, Inc., Bellefonte, Pa.

Table I—Average Solubilities of Three Fluorocarbon Propellants in Various Media

	Water[a]	Water or Normal Saline	Human Plasma	Human Blood	Human[b] Serum	Cyclohexane	Chloroform
Trichloromonofluoromethane	0.19	0.22	0.76	0.87	0.9	75.4	73.3
Dichlorodifluoromethane	0.055	0.060	0.085	0.15	0.2	22.4	14.6
Dichlorotetrafluoroethane	0.019	0.020	0.22	0.15	0.2	35.1	19.3

[a] Values calculated from literature data. See text for calculation. [b] Obtained from Ref. 5.

Table II—Partition Coefficients of Three Fluorocarbon Propellants in Cyclohexane–Water and Chloroform–Water Systems

Fluorocarbon Propellants	*P*(Cyclohexane–Water)	*P*(Chloroform–Water)
Trichloromonofluoromethane	343	333
Dichlorodifluoromethane	373	243
Dichlorotetrafluoroethane	1755	965

the solubilities of trichloromonofluoromethane and dichlorodifluoromethane in serum were much higher when fibrinogen and other clotting factors were removed from the plasma. The data of dichlorotetrafluoroethane from the two studies, however, appear to be in reasonable agreement.

The solubility of a volatile compound in blood is one of the most important factors affecting the rate of absorption after inhalation. Both dichlorodifluoromethane and dichlorotetrafluoroethane had the same solubility in blood, which was approximately one-sixth of that of trichloromonofluoromethane. A similar relationship also existed for the early absorption rates of these propellants in humans. The data in Fig. 2 from Ref. 5 showed that both dichlorodifluoromethane and dichlorotetrafluoroethane were absorbed at the same rate and that the absorption rate of trichloromonofluoromethane in the first 40 sec was approximately five to six times higher than the other two propellants.

The low solubilities of the three propellants studied in water, normal saline, human plasma, and human blood indicate that special precautions must be exercised in handling these chemicals to prevent any loss through evaporation. This is especially important for dichlorodifluoromethane and dichlorotetrafluoroethane. Paterson *et al.* (4) attributed the low or negligible blood levels of these two propellants in humans after inhalation to the possible rapid loss of the propellants from the blood samples and recommended that special techniques of collection were needed. Although the solubilities of the three propellants in organic solvents such as chloroform and cyclohexane (Table I) are markedly higher, the possible loss to the head space or gaseous phase from these media cannot be ignored, particularly when the ratio of the volume of the gaseous phase to that of the liquid phase is high. For example, a 49% loss to the head space at equilibrium may result if 1 ml of dichlorodifluoromethane in chloroform is contained in a sealed 15-ml capacity bottle. In the GC analysis of propellants in blood, if the peak height response from the extracted fluid is compared directly with standards that have not been subjected to such an equilibration with the head space, a significant error may be anticipated. It seems that such an error might have been made in a previous study (12) using chloroform for extraction from blood samples. Unfortunately, the analytical procedure reported in that study was not detailed enough to permit one to make a definite conclusion.

The partition coefficients of the three propellants in cyclohexane-water and chloroform-water systems are shown in Table II. These were calculated by dividing the solubility in the organic solvent by that in water. The higher partition coefficients of dichlorotetrafluoroethane in both systems indicate that it may have a larger volume of distribution in the body due to its accumulation in lipoid tissues and longer biological half-life based on the terminal phase of the blood concentration–decay curve (13–15). The limited data of the human study by Morgan *et al.* (5), which showed a slightly smaller elimination rate (smaller retention decay in their Fig. 3) between 10 and 30 min after inhalation for dichlorotetrafluoroethane than for trichloromonofluoromethane, tend to support such a contention.

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[x] To whom inquiries should be directed.