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Fluorocarbon Aerosol Propellants VIII: Solubility of Trichloromonofluoromethane in Dog Blood and Tissue Homogenates

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Abstract □ The solubility of trichloromonofluoromethane in dog blood and tissue homogenates was measured indirectly using the head-space method at 37°. These values, except that in dog blood, were used to estimate the solubility in the whole tissues or organs. In most cases, the solubility so obtained was independent of equilibrium concentration. However, a considerable concentration dependence for the solubility was observed in dog heart and kidney. The highest solubility found was in dog fat *versus* air, *i.e.*, 45.6, which is almost 220 times higher than the solubility in water or normal saline. Fat solubilization and specific binding interactions appeared to be the two major factors in determining the solubility. The pharmacokinetic implications of such findings are discussed.

Keyphrases □ Fluorocarbon aerosol propellants—solubility of trichloromonofluoromethane in dog blood and tissue homogenates □ Aerosol propellants—solubility of trichloromonofluoromethane in dog blood and tissue homogenates □ Propellants—solubility of trichloromonofluoromethane in dog blood and tissue homogenates □ Trichloromonofluoromethane—solubility in dog blood and tissue homogenates

The wide use of fluorocarbon aerosol propellants in various household, cosmetic, and medicinal pressurized packages recently prompted extensive studies on their possible adverse effects on the cardiovascular system (1–10), enzyme activities (7, 11–13), mutation (14), and ozone concentrations in the stratosphere (15, 16). The high apparent volume of distribution in dogs (17–19) and the moderate to extensive binding with both bovine and human albumins (20–23) of the three most commonly used fluorocarbon propellants prompted the investigation of their solubilities in various tissues and organs. The information obtained from such an investigation is essential to the construction of a physiologically based pharmacokinetic model as reported for barbiturates (24) and methotrexate (25). The results of the solubility study of trichloromonofluoromethane in blood and tissue homogenates of dogs are reported in this article.

For a gaseous or volatile compound, the solubility is commonly defined as the ratio of its concentration in the liquid phase to its concentration in the gaseous phase at the equilibrium state (19, 26, 27). Such a definition is analogous to that of the partition coefficient, P, which has been conventionally used to describe the distribution of a nonvolatile compound between two immiscible liquid phases. These two terms are used interchangeably in this article.

EXPERIMENTAL

Preparation of Samples—Three male mongrel dogs, 17–20 kg, were sacrificed and their tissues were separated, individually wrapped, and kept in a freezer. Prior to the homogenization of the lean tissue, all conspicuous attached fatty tissues were removed and the "clean" tissue was weighed and chopped. Then for each gram of the prepared tissue, 4 ml of chilled sodium chloride injection USP[1] was added and the mixture was homogenized in a blender[2].

The stock bottle[3] of trichloromonofluoromethane[4] (bp 23.82°) was prepared within a month of the experiment to minimize the rate of loss due to storage (28). The bottle had a nominal volume of 500 ml and a fluorocarbon concentration in the 0.64–0.96-mg/ml range.

Procedure—For the study of the partition coefficient of the propellant in each tissue, four pairs of 60-ml serum bottles were prepared. Each pair consisted of an empty control and a sample bottle into which 40 ml of the tissue homogenate was introduced with a 50-ml glass syringe. All bottles were sealed with lacquer-coated stoppers[5] and aluminum caps. They were then immersed in a water bath preset at 37° and shaken mechanically for 15 min.

This step was followed immediately by releasing the extra pressure developed in the bottles by inserting a syringe needle for a moment. Then a calculated amount of air was withdrawn from these bottles, which were expected to show significant pressure

[1] Sodium chloride injection USP, McGaw Laboratories, Division of American Hospital Supply Corp., Glendale, CA 91201
[2] Waring blender, Scientific Products, McGaw Park, IL 60085
[3] Wheaton serum bottle, Wheaton Scientific, Division of Wheaton Industries, Millville, NJ 08332
[4] Freon 11, E. I. du Pont de Nemours and Co., Wilmington, Del.
[5] Lacquer-coated rubber stopper, West Co., Phoenixville, Pa.

change due to the later introduction of the stock propellant. Both 50-μl and 2.5-ml gastight syringes[6] were used in delivering the propellant into the bottles so that a wide range of concentrations could be effected with ease. Each pair of bottles (sample and control) received essentially the same amount of the stock propellant.

Equilibration of the propellant with the tissue homogenates was approached by shaking the bottles for 90 min. The propellant concentration in the head space was then measured by GC under the previously reported conditions (23, 29). Since the concentrations to be measured were mostly above the GC linear response, dilution of the propellant in the gas phase was routinely done using the following precautions. Preliminary GC analysis was made to predict the concentration to be measured within a few hundred percent. In the final analysis, the amount withdrawn from the head space was injected into a presealed clean serum bottle (either 5- or 60-ml size as required). A second clean syringe must be used to flush the propellant in this dilution bottle and to take a sample for GC analysis. This syringe must be reserved only for such use. If, by accident, this syringe was used with the same propellant at 10-fold or higher concentration, then the rate of desorbing the propellant from this syringe would be so slow that it would forbid its further use for GC injection with accuracy for a long period. This phenomenon of adsorption and desorption in the syringe was also observed for dichlorodifluoromethane and dichlorotetrafluoroethane.

The specific gravity for a given tissue was determined by its weight and the volume of deionized water it displaced from a stoppered serum bottle.

Calculations—The partition coefficient of a tissue homogenate *versus* the air ($P_{t.h.}$) is determined indirectly using the head-space method (29, 30). For convenience in the present study, the equation can be rewritten in the following form:

$$P_{t.h.} = \frac{C_0}{C_h}(V_r + 1) - V_r \qquad \text{(Eq. 1)}$$

where C_0 = concentration of the fluorocarbon in the control bottle, C_h = concentration of the fluorocarbon in the head space of the sample bottle, and V_r = ratio of the volume of the gas phase to that of the homogenate phase.

To estimate the partition coefficient of the propellant in tissue *versus* air, the interaction due to the dispersion and dissolution of the whole tissue is neglected. Accordingly, the conservation of the propellant in the bottle leads to the following equation:

$$W = C_h V_h + C_t V_t + C_s V_s \qquad \text{(Eq. 2)}$$

where W = total weight of the fluorocarbon in the bottle; and V_h, V_t, and V_s = volume of the head space, the tissue, and the normal saline, respectively.

By dividing Eq. 2 by C_h and substituting the term C_t/C_h by P and C_s/C_h by P_s, the following equation can be derived:

$$P = \frac{W - C_h(P_s V_s + V_h)}{C_h V_t} \qquad \text{(Eq. 3)}$$

where P and P_s are the partition coefficients in tissue and in normal saline, respectively. This equation is essentially the same as that given by Larson *et al.* (27), except that in their paper the corresponding term W was misinterpreted as the volume. Equation 3 can be reduced to:

$$P = \frac{P_{t.h.} - P_s f_s}{f_t} \qquad \text{(Eq. 4}a\text{)}$$

where:

$$f_s = \frac{V_s}{V_s + V_t} \qquad \text{(Eq. 4}b\text{)}$$

$$f_t = 1 - f_s \qquad \text{(Eq. 4}c\text{)}$$

The equilibrium concentration in the tissue was calculated, after P was determined, according to the definition of P, namely:

$$C_t = PC_h \qquad \text{(Eq. 5)}$$

In the calculation of the partition coefficient of the propellant in tissue *versus* air, Eq. 1 was used at first to calculate the partition coefficient of the propellant in the tissue homogenate ($P_{t.h.}$). Then P was calculated according to Eq. 4*a*, where P_s was derived in the same way as $P_{t.h.}$. The value of P_s was 0.208. The specific gravities listed in Table I were used to evaluate f_s and f_t in Eq. 4*a*. The partition coefficient of the propellant in tissue *versus* blood was calculated simply by dividing the solubility in tissue by the solubility in blood.

[6] Hamilton Co., Reno, Nev.

Table I—Partition Coefficients of Trichloromonofluoromethane in Dog Blood and Tissues and the Specific Gravities of the Tissues

Tissue	Equilibrium Concentration[a], C_t, μg/ml	Partition Coefficient, P, C_t/C_g	Partition Coefficient, Tissue / Blood	Specific Gravity
Liver	0.014–6.6	1.64	1.09	1.076
Heart	1–2[b]	1.02	0.68	1.051
Kidney	1–2[b]	1.20	0.80	1.033
Brain	1.72–82.6	1.75	1.17	1.040
Lung	0.77–34.2	0.54	0.36	0.823
Spleen	1.8–33.0	1.61	1.07	1.065
Muscle	1.3–47.9	0.81	0.54	1.028
Fat	6.0–212	45.6	30.4	0.947
Blood	1.2–6.6	1.50	—	—

[a] Including both bound and free form of the propellant. [b] Estimated from Figs. 1 and 2.

RESULTS AND DISCUSSION

The partition coefficients for trichloromonofluoromethane in dog blood and most tissue homogenates at 37° were approximately constant over a wide range of equilibrium concentrations (Table I). Each value is an average of three or four test results. For the dog heart and kidney, the partition coefficients were considerably concentration dependent over the similar range of concentrations, and the average results of three replicate studies from three dogs were plotted in Figs. 1 and 2, respectively.

Recently, the binding interactions of the propellant trichloromonofluoromethane with human and bovine albumin were studied along with two other propellants (20–23). The partition coefficients of the propellants in the aqueous 5% human and bovine albumin solutions were highly dependent on the propellant concentration, indicating that specific binding sites are available on these protein molecules. Similarly, such specific interactions appeared to exist in the tissue of dog heart and kidney, in which the partition coefficients of trichloromonofluoromethane are higher at lower equilibrium concentrations. The results obtained from the present study may not truly reflect the *in vivo* interactions between the propellant and tissues or organs due to the homogenization of the samples.

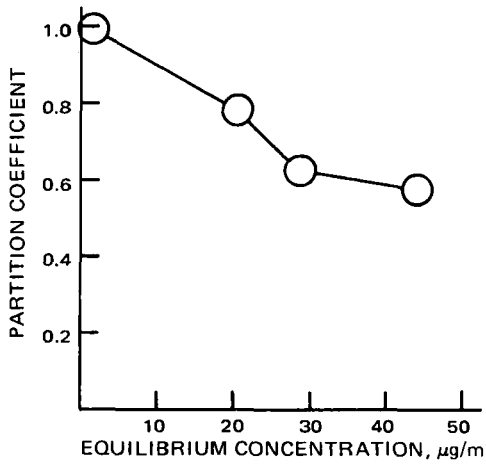

Figure 1—*Partition coefficient of trichloromonofluoromethane in dog heart* versus *air as a function of the equilibrium concentration in the heart tissue.*

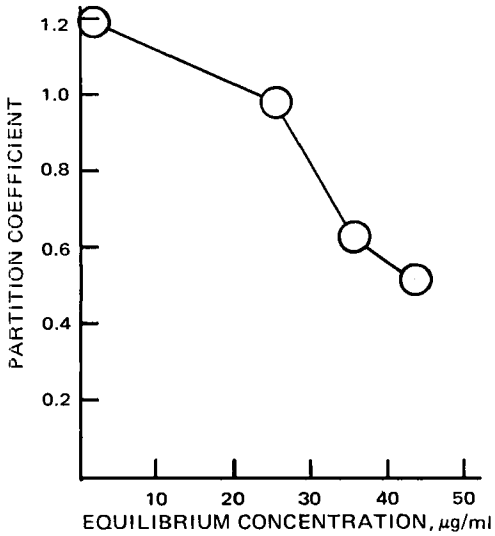

Figure 2—*Partition coefficient of trichloromonofluoromethane in dog kidney* versus *air as a function of the equilibrium concentration in the kidney tissue.*

In a study of arterial blood levels of fluorocarbons in asthmatic patients following use of pressurized aerosols (6), the dog heart muscle–human blood partition coefficient for trichloromonofluoromethane at 37° was determined to be 1.9. This value was derived at an equilibrium concentration in the heart probably slightly above 10 μg/ml. Accordingly, the solubility of this fluorocarbon in the dog heart is estimated to be 1.71 (*i.e.*, 1.9 × 0.9). No equilibrium concentration dependence was reported for such solubility. In the present study the solubility of trichloromonofluoromethane in dog heart was found to be concentration dependent (Fig. 1), and its value is 0.9 at the equilibrium concentration of 10 μg/ml. The discrepancy in the value of solubility may be attributed to the difference in dog species. Large variability of propellant solubility in blood and plasma among different animals was reported (18). In light of such differences, the simulation of the myocardial level of fluorocarbon in humans based on dog heart cannot be appropriate.

The partition coefficient of trichloromonofluoromethane in dog fat was calculated to be 45.6, the highest value obtained among all specimens studied. It is almost 220 times higher than that in normal saline or water, about 84 times higher than that in dog lung, and about 56 times greater than that in dog muscle. The relatively large solubility of the propellant in dog fat may be expected from its physical–chemical properties such as being nonionic, lipophilic, and highly soluble in cyclohexane (20, 23).

The partition coefficients of dog blood and tissues were greater than those in water or normal saline. This finding may be explained by the following two reasons:

1. The presence of a small amount of fat in the specimen can enhance the solubilization of the propellant. The fat contents of dog liver, lung, spleen, muscle, and heart were reported by Brodie *et al.* (31) and ranged from 1.6 to 3.0%. Thus, by solubilization in fat alone, the partition coefficients of the propellant in these tissues are expected to be at least from 0.73 to 1.37; these values are not too far from the values determined in this study in view of the possible biological variance.

2. The specific interaction (*e.g.*, protein binding and complexation) and other nonspecific interaction such as solubilization by surfactant also can increase the solubility of this propellant.

As mentioned, the specific interaction such as protein binding could explain the phenomenon of a concentration-dependent partition coefficient as observed in dog heart and kidney tissue. Such an effect of the specific interaction may also be observed from a complexation or hydrophobic interaction with other tissue components. When the binding sites are saturated or the binding capacity of a tissue is very small, the phenomenon of the concentration dependence of partition coefficient may not be easily observed.

The previous pharmacokinetic study (19) on the three dogs used in the present tissue solubility study showed that, on the average, the apparent volume of distribution of trichloromonofluoromethane in terms of blood concentration, estimated after the attainment of a pseudo-distribution equilibrium, was about six times their body weight. Since the partition coefficients of this propellant in all tissues studied except fat are fairly close to, or less than, that in blood, one can reasonably assume that the observed higher apparent volume of distribution is primarily due to its concentration or accumulation in fat tissues. The uptake by the fat tissues probably accounts for the existence of the second (deep) tissue compartment in the disposition of the propellant in dogs (19). Subjects with higher fat content also can conceivably accumulate more of this type of compound and have a longer biological half-life. The toxicological implications of this study remain to be explored.

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