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Partition Coefficients of Low-Molecular-Weight Volatile Chemicals in Various Liquids and Tissues

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Partition Coefficients of Low-Molecular-Weight Volatile Chemicals in Various Liquids and Tissues. GARGAS, M. L., BURGESS, R. J., VOISARD, D. E., CASON, G. H., AND ANDERSEN, M. E. (1989). *Toxicol. Appl. Pharmacol.* **98,** 87–99. Partition coefficients are required for developing physiologically based pharmacokinetic models used to assess the uptake, distribution, tabolism, and elimination of volatile chemicals in mammals. A gas-phase vial equilibration technique is presented for determining the liquid:air and tissue:air partition coefficients for low-molecular-weight volatile chemicals. This technique was developed from two previously described medium:air methods, relied solely on measurement of chemical concentration in the gas phase, and, compared to earlier work, extends the range of chemicals and tissues examined. Partition coefficients were determined with 0.9% saline, olive oil, and blood, liver, muscle, and fat tissues from rats for 55 compounds. Human blood:air coefficients were determined for 36 compounds and several blood:air values were also determined in the mouse and for one compound in the hamster. An approach is described for predicting the tissue solubilities of untested compounds based on oil:air and saline:air coefficients using regression analyses. A similar approach is used to model fat:air coefficients in terms of oil:air values and to model human blood:air coefficients in terms of rat blood:air coefficients.

A partition coefficient for a given chemical is the ratio of concentrations achieved between two different media at equilibrium. Blood:air and tissue:air or tissue:blood coefficients are required to describe the pulmonary uptake and distribution of volatile chemicals in mammalian systems (Fiserova-Bergerova *et al.,* 1984; Fiserova-Bergerova and Diaz, 1986; Andersen, 1982, 1983) and constitute an integral component in the development of physiologically based pharmacokinetic (PB-PK) models (Ramsey and Andersen, 1984; Gargas *et al.,* 1986a,b; D'Souza *et al.,* 1988). Our laboratory has been very active developing PB-PK models over the past several years and we have required solubility information for a variety of volatile chemicals. Several existing techniques for determining partition coefficients were considered, including a headspace vial-equilibration technique for blood, oil, and water (Sato and Nakajima, 1979a), a similar technique that included tissue homogenates (Thomas, 1975; Fiserova-Bergerova *et al.,* 1984), and a method based on the determination of purging rate (Van Rees, 1974).

The method chosen for this present work is a modified version of the vial-equilibration technique (Sato and Nakajima, 1979a) that also includes tissue homogenates prepared in 0.9% saline solution (Fiserova-Bergerova *et al.,* 1984). These techniques were attractive because no elaborate experimental apparatus was required and the partition coefficient could be determined by measuring headspace concentrations in test vials and appropriate

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reference vials, with no requirement for measuring chemical concentration in the test medium.

This paper describes the technique used to determine partition coefficients and presents the results obtained to date for a group of 55 low-molecular-weight, volatile compounds. The liquid:air and tissue:air partitions were determined in 0.9% saline, in olive oil, and in blood, liver, muscle, and fat tissues from rats. Human blood:air values were determined for 36 of the compounds and several blood:air values were also determined for the mouse and for one chemical in the hamster. Relationships between rat blood:air and human blood:air partition coefficients and between olive oil:air and fat:air partition coefficients, and modeling of the various tissue:air and blood:air coefficients in terms of their saline and oil:air values, were also examined using linear regression analyses.

METHODS

Animals and chemicals. The animals utilized in this study were male Fischer 344 rats (strain designated CDF [F-344] CrlBr), male and female B6C3F1 mice (strain B6C3F1/Crl Br), male CD-1 mice (strain Crl:CD-1 [ICR] Br), and male golden Syrian hamsters (strain Lak: LVG [SYR]) (all from Charles River Breeding Laboratory, Kinston, NY) Rats weighed between 200 and 300 g, mice weighed between 25 and 35 g, and the hamster weighed in the range 120–140 g. All animals were housed in a portable laminar air flow enclosure system with a 12-hr on, 12-hr off light cycle. All animals had free access to commercial food (Purina) and tap water in the holding facility. The 55 chemicals utilized for the partition coefficient measurements were of the highest purity available (>98%) with the exception of pentachloroethane (96%).

Test media. The liquids and tissues utilized were: 0.9% NaCl (saline) solution, reagent-grade olive oil (Fisher Scientific Company, Fairhaven, NJ), heparanized rat, mouse, hamster, and human blood, and rat liver, muscle (thigh), and fat (epididymal and perirenal) tissues. Tissues were prepared as homogenates in saline solution in a 1:3 tissue weight:saline volume ratio (assuming a tissue density of 1.0 g/ml). The saline:air partition coefficient determinations were necessary to account for the amount of partitioning due to saline in the tissue homogenates and the oil:air values were used for comparison with the fat:air coefficients. The saline:air and oil:air partition coefficients were also used as independent variables for modeling the blood and tissue:air values in multiple linear regression analyses.

Equipment. Liquid scintillation vials (Kimble, Division of Owens, Illinois, Toledo, OH) with an average measured volume (water displacement) of 24.65 ± 0.01 (SE) ml were used as the equilibration vessels. The vial caps were modified by removing the manufacturer-supplied liner, drilling a 4-mm-diameter hole, and placing a Teflon-coated rubber septum (Supelco Inc., Bellefonte, PA; 2 cm in diameter) in the cap. The septum provided an airtight seal and the hole provided ready access to the air phase through a gastight syringe (Hamilton Co., Reno, NV) with sideport needle. Chemical was introduced into the vials as a vapor by injecting 1 ml of atmosphere from a gas sampling bag (SKC Inc., Eight Four, PA) containing an air concentration of between 5000 and 10,000 ppm. Starting concentrations in the vials were approximately 200–400 ppm. A temperature-controlled Vortex evaporator (Haake/Buchler Instrument Inc., Saddlebrook, NJ) was used to agitate and incubate (37°C) the vials until equilibrium between the headspace air and test material was achieved. This instrument consisted of an aluminum heating block with spaces for up to 20 vials. This aluminum block was temperature controlled and mounted on a rotating stage. It was found that the shortest time to equilibrium could be achieved if the vials were vortexed at 200 to 300 rpm. The headspace chemical concentrations were analyzed by gas chromatography (Hewlett Packard, Avondale, PA) utilizing a flame ionization detector and, in one case, an electron capture detector (for hexachloroethane). The gas chromatograph temperature conditions and carrier gas flow rates varied between chemicals, but adjustments were made ensuring retention times of between 1.0 and 3.0 min. Standard curves were prepared for each chemical to ensure linear integrator responses and were periodically checked during the time a particular chemical was under study.

Procedure. Liquids and tissues for analysis by this technique can be divided into two classes: those requiring saline in the reference vials and those with no diluent liquid in the reference vials. Heparanized blood, saline, and olive oil are those that require empty reference vials; the three tissue homogenates require saline references. Blood, saline, and oil is analyzed as follows. Three vials are used for each type of medium, including three empty reference vials. Two milliliters of blood, 2 ml of saline, and 0.2 ml of oil are pipetted into each of the test vials. The three test and three empty reference vials are then capped and placed in the 37°C incubator/mixer. All vials are allowed to reach thermal equilibration for approximately 15 min. Excess pressure is relieved through a sideport needle, open to room air and inserted through the septum. A volume equal to the volume of test chemical to be injected is then removed (usually 1 ml). This procedure ensures an internal vial pressure that approximates

TABLE 1

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—METHANES

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
1	Methyl chloride	2.47 ± 0.16[a]	0.88 ± 0.08	8.57 ± 0.22	13.5 ± 0.4	3.47 ± 0.25	0.97 ± 0.38
2	Dichloromethane	19.4 ± 0.8	5.96 ± 0.71	131 ± 7	120 ± 6	14.2 ± 1.2	7.92 ± 1.77
3	Chloroform	20.8 ± 0.1	3.38 ± 0.09	402 ± 12	203 ± 5	21.1 ± 1.5	13.9 ± 1.9
4	Carbon tetrachloride	4.52 ± 0.35	0.35 ± 0.03	374 ± 11	359 ± 11	14.2 ± 1.0	4.57 ± 0.59
5	Difluoromethane	1.60 ± 0.10	1.31 ± 0.05	4.76 ± 0.75	1.43 ± 0.31	2.75 ± 0.39	1.44 ± 0.25
6	Fluorochloromethane	5.08 ± 0.06	3.08 ± 0.07	22.3 ± 1.4	15.4 ± 1.0	3.44 ± 0.27	2.46 ± 0.52
7	Bromochloromethane	41.5 ± 0.9	8.65 ± 0.28	361 ± 9	325 ± 3	29.2 ± 0.5	11.1 ± 1.8
8	Dibromomethane	74.1 ± 1.5	14.4 ± 0.4	957 ± 39	792 ± 14	68.1 ± 1.4	40.5 ± 2.0
9	Chlorodibromomethane	116 ± 4	7.34 ± 0.42	2683 ± 152	1917 ± 165	126 ± 7.1	55.6 ± 0.7

[a] Values are means ± SE, where n = 3–15 samples. The range of sample (n) resulted from additional determinations performed in an effort to reduce the standard errors and/or to ensure equilibrium conditions.

the ambient room pressure after chemical is introduced. One milliliter of chemical atmosphere from a previously prepared gas sampling bag is injected into each vial and the entire set of vials is vigorously mixed for a 1-hr equilibrium period. A duplicate set of vials are prepared and allowed to incubate for 3 hr. At the end of the 1- and 3-hr incubations, 1 ml of headspace from each vial is removed and the chemical concentrations are determined by gas chromatography. Partition coefficients are determined using the equation

$$P_i = \frac{C_{ref}(V_{vial}) - C_i(V_{vial} - V_i)}{C_i(V_i)} \quad (1)$$

where P_i is the partition coefficient (i = blood, saline, or oil), C_{ref} is the chemical concentration in the headspace of the reference vial, C_i is the headspace chemical concentration of the test vial, and V_{vial} and V_i are the volumes of empty vial (24.65 ml) and test liquid, respectively. This equation is a simplified version of Eq. (3) from Sato and Nakajima (1979a). The partition coefficients from the 1- and 3-hr incubation periods are compared, and if no significant increase is noted at 3 hr, equilibrium is assumed to have occurred. If an increase is observed at 3 hr, the experiment is repeated with sampling at 2 and 4 hr or until equilibrium is achieved, as indicated by two consecutive time points with no significant difference in partition values.

The tissue homogenates (liver, muscle, and fat) are prepared in 1:3 weight:saline volume ratios and are analyzed in a similar manner. Two milliliters of each homogenate is pipetted into each of three vials. This ratio results in test vials containing 0.5 ml of tissue and 1.5 ml of saline. The reference vials for this homogenate ratio require 1.5 ml of saline. Other homogenate ratios can be used, providing the reference vial contains an equivalent volume of saline. For example, if tissues are homogenized in a 1:4 weight:saline volume ratio and 2.0 ml of homogenate is used, the reference vial would require 1.6 ml saline. In this case, the test vial would contain 0.4 ml of tissue and 1.6 ml of saline. All vials for this analysis are treated as described above (i.e., temperature equilibration, venting, chemical introduction, incubation times, and headspace analysis). The partition coefficients are calculated by the equation

$$P_j = \frac{C_{ref}(V_{vial} - V_{sal}) - C_j(V_{vial} - V_{sal} - V_j) + (C_{ref} - C_j)P_{sal}V_{sal}}{C_j(V_j)} \quad (2)$$

where P_j is the partition coefficient (j = liver, muscle, or fat), V_{sal} is the saline volume (1.5 ml if the homogenate ratio is 1:3), P_{sal} is the saline:air partition coefficient as described earlier and calculated using Eq. (1), C_{ref} and C_j are the headspace concentrations in the reference and test vials, respectively, and V_{vial} and V_j are the volumes of the empty vial (24.65 ml) and tissue (0.5 ml if the homogenate ratio is 1:3), respectively. This equation is the same as Eq. (3) of Sato and Nakajima (1979a). Note that Eq. (2) reduces to Eq. (1) when the reference vial is empty (i.e., V_{sal} = 0.0 ml).

RESULTS

The rat tissue:air and liquid:air partition coefficients were determined for six general classes of lower-molecular-weight chemicals (Tables 1–6) and for four chemicals not belonging to any one of these classes (Table 7). Of the 55 chemicals studied, human blood:air values were determined for 36 of the compounds (Table 8). In addition to blood:air

TABLE 2

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—ETHANES

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
10	Chloroethane	4.08 ± 0.39	1.09 ± 0.06	38.9 ± 3.1	38.6 ± 0.7	3.61 ± 0.32	3.22 ± 0.68
11	1,1-Dichloroethane	11.2 ± 0.1	2.45 ± 0.04	186 ± 7	164 ± 4	10.8 ± 0.5	5.12 ± 0.48
12	1,2-Dichloroethane	30.4 ± 1.2	11.4 ± 0.1	366 ± 8	344 ± 5	35.7 ± 1.6	23.4 ± 1.4
13	1,1,1-Trichloroethane	5.76 ± 0.50	0.75 ± 0.07	295 ± 22	263 ± 12	8.6 ± 0.6	3.15 ± 0.33
14	1,1,2-Trichloroethane	58.0 ± 1.1	13.3 ± 0.3	1776 ± 26	1438 ± 58	73.1 ± 0.8	22.9 ± 0.8
15	1,1,1,2-Tetrachloroethane	41.7 ± 1.0	3.53 ± 0.23	2686 ± 51	2148 ± 82	88.2 ± 1.8	39.5 ± 2.5
16	1,1,2,2-Tetrachloroethane	142 ± 6	23.4 ± 2.0	6358 ± 402	3767 ± 93	196 ± 12	101 ± 10
17	Pentachloroethane[a]	104 ± 3	2.32 ± 0.35	6689 ± 471	4118 ± 209	260 ± 11	72.4 ± 2.9
18	Hexachloroethane	62.7 ± 2.1	0.66 ± 0.21	5015 ± 318	3321 ± 193	369 ± 17.5	75.0 ± 0.9
19	1,2-Dibromoethane	119 ± 7	17.3 ± 0.8	1276 ± 91	1219 ± 50	119 ± 4	45.6 ± 3.3
20	1-Bromo-2-chloroethane	52.7 ± 3.5	8.91 ± 0.56	569 ± 23	959 ± 39	42.8 ± 3.3	25.4 ± 3.1
21	1,1,1-Trifluoro-2-chloroethane	1.27 ± 0.06	0.42 ± 0.04	24.0 ± 2.5	21.2 ± 0.6	1.84 ± 0.14	1.23 ± 0.14
22	1,1,1-Trifluoro-2-bromo-2-chloroethane	5.26 ± 0.13	0.50 ± 0.05	198 ± 4	182 ± 5	7.62 ± 1.20	4.46 ± 0.29

[a] The 0.9% saline solution used for determining saline:air ratios and for preparing tissue homogenates was acidified with HCl to a pH of 3.5. See the Discussion for a further explanation.

values for dichloromethane in the male F. 344 rat, human, and B6C3F1 male mouse. blood:air values were also determined in the male hamster (22.5 ± 0.8), female B6C3F1 mouse (7.56 ± 0.34), and male CD-1 mouse (8.29 ± 0.32). A trichloroethylene blood:air partition coefficient was also determined in the male CD-1 mouse (15.3 ± 0.54).

Olive oil:air partition coefficients for 20 of the compounds presented in this work compared very well (Fig. 1) with those reported by Sato and Nakajima (1979a,b). Human blood: air partition coefficients reported here were also compared with the results of Sato and Nakajima (1979a,b) for 20 compounds (Fig. 2) and a comparison of 7 human blood:air values was also possible (Fig. 3) with data reported by Fiserova-Bergerova and Diaz (1986). In both cases, the present results were very comparable with earlier reported values.

The feasibility of estimating rat fat:air partitions from olive oil:air measurements was tested by linear regression analysis. Log fat: air values, $\log(P_{fat})$, were modeled in terms of the corresponding log oil:air coefficients, $\log(P_{Oil})$. The resulting equation demonstrates the excellent correlation

$$\log(P_{fat}) = 0.920(\pm 0.030)\log(P_{oil}) + 0.136(\pm 0.083)$$

$$r^2 = 0.946 \quad s = 0.187 \qquad (3)$$

where $n = 55$, s is the root-mean-square error of the fit, r^2 is the square of the correlation coefficient, and $p < 0.0001$ (the probability that the fit occurred by chance). Values in parentheses are the standard errors about the coefficients.

A similar analysis was performed for estimating the log of the human blood:air coefficients, $\log(P_{HBL})$, in terms of log rat blood: air values, $\log(P_{blood})$. The equation

$$\log(P_{HBL}) = 1.014(\pm 0.037)\log(P_{blood}) - 0.232(\pm 0.051)$$

$$r^2 = 0.957 \quad s = 0.132 \qquad (4$$

also exhibits excellent correlation with $n = 3$

TABLE 3

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—PROPANES

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
23	1-Chloropropane	5.21 ± 0.06	1.04 ± 0.01	105 ± 2	118 ± 2	5.18 ± 0.38	2.08 ± 0.66
24	2-Chloropropane	3.10 ± 0.17	0.82 ± 0.09	69.9 ± 3.5	68.4 ± 2.0	3.15 ± 0.24	2.04 ± 0.48
25	1,2-Dichloropropane	18.7 ± 0.5	2.75 ± 0.11	428 ± 30	499 ± 30	24.8 ± 2.4	12.0 ± 1.1
26	*n*-Propyl bromide	11.7 ± 0.4	1.44 ± 0.12	272 ± 8	236 ± 6	8.17 ± 0.62	4.21 ± 0.32
27	Isopropyl bromide	5.95 ± 0.14	1.08 ± 0.04	164 ± 5	158 ± 5	4.41 ± 0.34	4.12 ± 0.35
28	1-Nitropropane	223 ± 10	127 ± 4	1062 ± 21	506 ± 33	153 ± 17	28.9 ± 6.1
29	2-Nitropropane	183 ± 12	98.3 ± 5.4	640 ± 16	155 ± 4	62.4 ± 1.4	29.1 ± 3.3

and $p < 0.0001$. A plot of human blood:air versus rat blood:air coefficients (Fig. 4) and comparisons of the data (Table 8) reveal that rat values tend to be 1.5 to 2.0 times higher than the corresponding human values in most instances. The line predicted (Fig. 4) using Eq. (4) runs below and approximately parallel to the line of unity.

Sato and Nakajima (1979b) reported an empirical relationship between blood:air partition coefficients and oil:air and water:air coefficients for a series of chlorinated compounds. They successfully modeled human blood:air coefficients in terms of contributions from oil:air and water:air values using linear regression techniques. It was of interest here to see if rat blood and tissue partition coefficients and the human blood:air values could be modeled as functions of the corresponding oil and saline:air values. This approach has previously been applied to a subset of 25 of the 55 compounds described in this report (Gargas *et al.*, 1988). The equations resulting from this present data set are

$$\log(P_{blood}) = 0.553(\pm 0.030)\log(P_{oil}) + 0.351(\pm 0.025)\log(P_{sal}) - 0.286(\pm 0.080)$$
$$n = 55 \quad r^2 = 0.928 \quad s = 0.179 \qquad (5)$$

$$\log(P_{liver}) = 0.730(\pm 0.036)\log(P_{oil}) + 0.128(\pm 0.030)\log(P_{sal}) - 0.550(\pm 0.097)$$
$$n = 55 \quad r^2 = 0.903 \quad s = 0.217 \qquad (6)$$

$$\log(P_{muscle}) = 0.644(\pm 0.038)\log(P_{oil}) + 0.180(\pm 0.032)\log(P_{sal}) - 0.725(\pm 0.104)$$
$$n = 55 \quad r^2 = 0.879 \quad s = 0.233 \qquad (7)$$

TABLE 4

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—ALIPHATICS

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
30	*n*-Hexane	2.29 ± 0.11	0.026 ± 0.009	155 ± 1	159 ± 2	5.2 ± 0.8	2.9 ± 0.6
31	*n*-Heptane	4.75 ± 0.15	0.18 ± 0.10	405 ± 3	379 ± 6	15.0 ± 0.7	4.20 ± 0.80
32	Cyclohexane	1.39 ± 0.09	<0.01	293 ± 2	235 ± 4	7.88 ± 0.59	1.03 ± 0.17
33	2,3,4-Trimethylpentane	3.75 ± 0.15	<0.01	662 ± 2	443 ± 20	18.8 ± 0.5	4.41 ± 0.35
34	2,2,4-Trimethylpentane	1.77 ± 0.12	<0.01	366 ± 12	293 ± 10	10.7 ± 0.2	3.30 ± 0.46
35	JP-10 (tricyclo[5.2.1.$0^{2,6}$]-decane)	62 ± 4	0.21 ± 0.07	12,970 ± 420	10,139 ± 239	554 ± 17	674 ± 19

TABLE 5

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—ETHYLENES

Compound number	Chemical	Blood	0.9% Saline	Oliver oil	Fat	Liver	Muscle
36	Vinyl chloride	1.68 ± 0.18	0.43 ± 0.04	24.4 ± 3.7	20.0 ± 0.7	1.60 ± 0.17	2.10 ± 0.45
37	1,1-Dichloroethylene	5.00 ± 0.19	0.35 ± 0.06	64.3 ± 3.4	68.6 ± 2.1	4.42 ± 0.30	2.05 ± 0.31
38	*cis*-1,2-Dichloroethylene	21.6 ± 2.0	3.25 ± 0.12	278 ± 6	227 ± 11	15.3 ± 1.1	6.09 ± 1.02
39	*trans*-1,2-Dichloroethylene	9.58 ± 0.94	1.41 ± 0.04	178 ± 6	148 ± 11	8.96 ± 0.61	3.52 ± 0.54
40	Trichloroethylene	21.9 ± 1.4	0.83 ± 0.30	553 ± 46	554 ± 21	27.2 ± 3.4	10.1 ± 2.7
41	Tetrachloroethylene	18.9 ± 1.1	0.79 ± 0.06	2134 ± 159	1638 ± 91	70.3 ± 9.0	20.0 ± 2.5
42	Vinyl bromide	4.05 ± 0.16	0.44 ± 0.06	56.0 ± 1.5	49.2 ± 1.3	3.33 ± 0.38	2.26 ± 0.13

$$\log(P_{fat}) = 0.927(\pm 0.031)\log(P_{oil}) - 0.032(\pm 0.026)\log(P_{sal}) + 0.120(\pm 0.083) \quad n = 55 \quad r^2 = 0.947 \quad s = 0.186 \qquad (8)$$

$$\log(P_{HBL}) = 0.581(\pm 0.055)\log(P_{oil}) + 0.332(\pm 0.039)\log(P_{sal}) - 0.599(\pm 0.150) \quad n = 36 \quad r^2 = 0.875 \quad s = 0.227. \qquad (9)$$

In all cases $p < 0.0001$, and as judged by r^2 values, all tissues were adequately modeled by this technique.

DISCUSSION

This work describes a modification and extension of two existing techniques for determining the solubility or partition coefficients of a diverse group of chemicals in a variety of liquids and tissues. Sato and Nakajima (1979a) used liquid chemical dissolved in water for delivery of chemical to the vials. In our hands, more reproducible results were obtained by introducing chemical to the vial headspace as a vapor. Following the approach of Fiserova-Bergerova *et al.* (1984), these techniques were extended to include tissues as test media, providing the solubility information required for developing PB-PK models for these classes of compounds.

The results indicate good correlation between this approach and the previous two methods for comparable compounds and serves as a validation for this present technique. Figure 1 shows the relationship between olive oil:air values for the present results and those reported by Sato and Nakaj-

TABLE 6

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—AROMATICS

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
43	Benzene	17.8 ± 0.3	2.75 ± 0.10	465 ± 5	499 ± 12	17.0 ± 1.3	10.3 ± 0.9
44	Chlorobenzene	59.4 ± 1.0	2.81 ± 0.07	2,188 ± 41	1,277 ± 43	86.1 ± 3.0	34.0 ± 3.9
45	Toluene	18.0 ± 1.0	1.75 ± 0.13	1,056 ± 38	1,021 ± 11	83.6 ± 5.8	27.7 ± 4.0
46	Styrene	40.2 ± 3.7	1.41 ± 0.47	3,548 ± 269	3,476 ± 73	139 ± 7	46.7 ± 3.9
47	*m*-Methylstyrene	192 ± 6	1.97 ± 0.28	14,706 ± 665	11,951 ± 692	327 ± 23	182 ± 10
48	*p*-Methylstyrene	234 ± 6	2.11 ± 0.30	13,942 ± 567	11,281 ± 972	324 ± 17	183 ± 8
49	*o*-Xylene	44.3 ± 2.0	2.65 ± 0.08	3,534 ± 208	1,877 ± 132	108 ± 7	51.5 ± 6.7
50	*m*-Xylene	46.0 ± 1.5	1.92 ± 0.12	3,245 ± 116	1,859 ± 93	90.9 ± 4.4	41.9 ± 5.7
51	*p*-Xylene	41.3 ± 3.5	1.77 ± 0.07	3,319 ± 96	1,748 ± 65	90.0 ± 4.3	38.4 ± 4.1

TABLE 7

RAT TISSUE:AIR AND LIQUID:AIR PARTITION COEFFICIENTS AT 37°C—OTHER COMPOUNDS

Compound number	Chemical	Blood	0.9% Saline	Olive oil	Fat	Liver	Muscle
52	Diethyl ether	12.2 ± 0.4	11.4 ± 0.4	55.6 ± 1.4	47.7 ± 3.9	6.82 ± 0.54	5.28 ± 0.54
53	Isoflurane	1.79 ± 0.13	0.56 ± 0.04	78.9 ± 9.1	98.1 ± 4.6	4.07 ± 0.20	1.60 ± 0.34
54	Allyl chloride	17.3 ± 0.6	2.06 ± 0.01	109 ± 5	101 ± 2	38.9 ± 4.5	11.0 ± 0.2
55	Isoprene	1.87 ± 0.10	0.21 ± 0.02	8.81 ± 0.15	72.0 ± 2.4	3.12 ± 0.87	2.04 ± 0.27

ima (1979a,b), and even though the correlation appears very good ($r^2 = 0.982$), the results of the previous technique exhibit a tendency to be somewhat higher (approximately 1.2–1.5 times) than those reported here as judged by the line with a slope of 1.0 (Fig. 1). Van Rees (1974) determined the olive oil:air partition coefficient for styrene to be 4100 by a purging rate technique, which is also slightly higher than the value of 3548 reported here (Table 6). It may be that these differences are attributable to the magnitude of the values measured and the corresponding increase in errors and/or to differences in the olive oil used. Nonetheless, the correlation can still be considered adequate. Comparisons of the human blood:air values (Figs. 2 and 3) do not exhibit any noticeable trends between the methods and both show adequate correlations ($r^2 = 0.979$ and 0.876, respectively).

Approximated values were reported for three saline:air partition coefficients (compounds 32, 33, 34; Table 4). For these compounds and others with very low aqueous solubility, it is possible that negative values can be computed using 2.0 ml of saline and applying Eq. (1). In these instances, increasing the volume (i.e., 5–10 ml) of the test substance is one way of alleviating this problem. Another approach would be to determine chemical concentrations both in the air phase and in saline, using extraction techniques that can be quite rigorous and time consuming. It has been our experience that progressively increasing the test volume works very well and is preferred. The approximated values reported here represent the sensitivity limit of the technique and indicate that this approach could not accurately detect the very small differences between test and reference vials found when testing these compounds. It is important to note, however, that saline:air partition coefficients are intended for use in Eq. (2) for calculating tissue values, and a very low coefficient or even zero simply means that the saline contribution to loss from the headspace in tissue homogenate vials will be minimal and will have little effect on the final tissue:air coefficients that are calculated.

The saline:air partition coefficient for pentachloroethane (Table 2) exhibited continued uptake of chemical for incubation periods over 6 hr, indicating an active uptake process in the test vial. The use of several other diluent liquids was attempted, including distilled water, 10 mM tris(hydroxymethyl)aminomethane buffer, and 0.9% saline solution, pH adjusted to 10.4 with 1.0 N NaOH. None of these liquids were found suitable, all demonstrated continued uptake of chemical to varying degrees. A 0.9% saline solution, pH adjusted to 3.5 with 1.0 N HCl, was found to reach equilibrium within 2 hr, with no continued uptake observed. This solution was employed as the diluent for the tissue homogenates used for pentachloroethane. It was also interesting to note that no active uptake process was found with rat blood. At this time we do not know the mechanism for reaction of pentachloroethane in saline and buffer solutions.

Active uptake into human blood was also observed for methyl chloride (Table 8). This

TABLE 8

HUMAN, MALE F-344 RAT, AND B6C3F1 MALE MOUSE BLOOD:AIR PARTITION COEFFICIENTS AT 37°C

Compound number[a]	Chemical	Human	F-344 rat[a]	B6C3F1 mouse
1	Methyl chloride	2.48 ± 0.23[b]	2.47 ± 0.16	—
2	Dichloromethane	8.94 ± 0.13	19.4 ± 0.8	5.79 ± 0.46
3	Chloroform	6.85 ± 0.51	20.8 ± 0.1	21.3 ± 1.2
4	Carbon tetrachloride	2.73 ± 0.23	4.52 ± 0.35	—
9	Chlorodibromomethane	52.7 ± 1.2	116 ± 4	—
10	Chloroethane	2.69 ± 0.20	4.08 ± 0.39	—
11	1,1-Dichloroethane	4.94 ± 0.24	11.2 ± 0.1	—
12	1,2-Dichloroethane	19.5 ± 0.7	30.4 ± 1.2	—
13	1,1,1-Trichloroethane	2.53 ± 0.13	5.67 ± 0.50	—
14	1,1,2-Trichloroethane	35.7 ± 0.4	58.0 ± 1.1	—
15	1,1,1,2-Tetrachloroethane	30.2 ± 1.3	41.7 ± 1.0	—
16	1,1,2,2-Tetrachloroethane	116 ± 6	142 ± 6	—
18	Hexachloroethane	52.4 ± 1.4	62.7 ± 2.1	—
20	1-Bromo-2-chloroethane	29.2 ± 2.1	52.7 ± 3.5	—
23	1-Chloropropane	2.85 ± 0.06	5.21 ± 0.06	—
24	2-Chloropropane	1.39 ± 0.29	3.10 ± 0.17	—
25	1,2-Dichloropropane	8.75 ± 0.50	18.7 ± 0.5	—
26	*n*-Propyl bromide	7.08 ± 0.40	11.7 ± 0.4	—
27	Isopropyl bromide	2.57 ± 0.15	5.95 ± 0.14	—
28	1-Nitropropane	187 ± 6	223 ± 10	—
29	2-Nitropropane	154 ± 17	183 ± 12	—
31	*n*-Heptane	2.85 ± 0.15	4.75 ± 0.15	—
32	Cyclohexane	1.41 ± 0.14	1.39 ± 0.09	—
34	2,2,4-Trimethylpentane	1.60 ± 0.17	1.77 ± 0.12	—
35	JP-10	52.5 ± 3.7	62 ± 4	—
36	Vinyl chloride	1.16 ± 0.08	1.68 ± 0.18	2.26 ± 0.15
38	*cis*-1,2-Dichloroethylene	9.85 ± 0.70	21.6 ± 2.0	19.5 ± 0.8
39	*trans*-1,2-Dichloroethylene	6.04 ± 0.38	9.58 ± 0.94	—
40	Trichloroethylene	8.11 ± 0.17	21.9 ± 1.4	—
41	Tetrachloroethylene	10.3 ± 1.1	18.9 ± 1.1	17.5 ± 0.4
42	Vinyl bromide	2.27 ± 0.16	4.05 ± 0.16	3.75 ± 0.45
43	Benzene	8.19 ± 0.10	17.8 ± 0.3	12.1 ± 0.3
44	Chlorobenzene	30.0 ± 0.3	59.4 ± 1.0	—
49	*o*-Xylene	34.9 ± 1.7	44.3 ± 2.0	—
50	*m*-Xylene	32.5 ± 1.6	46.0 ± 1.5	—
51	*p*-Xylene	44.7 ± 1.9	41.3 ± 3.5	—

[a] These values for rat are taken from Tables 1–7 and are reproduced here for ease of comparison with human blood:air values.

[b] The human blood:air value was determined on blood pretreated with diethyl maleate to inhibit a glutathione transferase reaction that was observed. See the Discussion for a further explanation.

active process was inhibited by the addition of 2.3 μmol diethyl maleate (DEM) per milliliter of blood. DEM depletes glutathione levels (Andersen *et al.*, 1980) and the active uptake process for methyl chloride is probably due to a glutathione *S*-transferase-catalyzed reaction. It is unlikely that this process was a non-enzyme-catalyzed glutathione conjugation because rat blood did not demonstrate this process, and one of the human volunteers had no continued uptake even though he had glutathione levels comparable to the

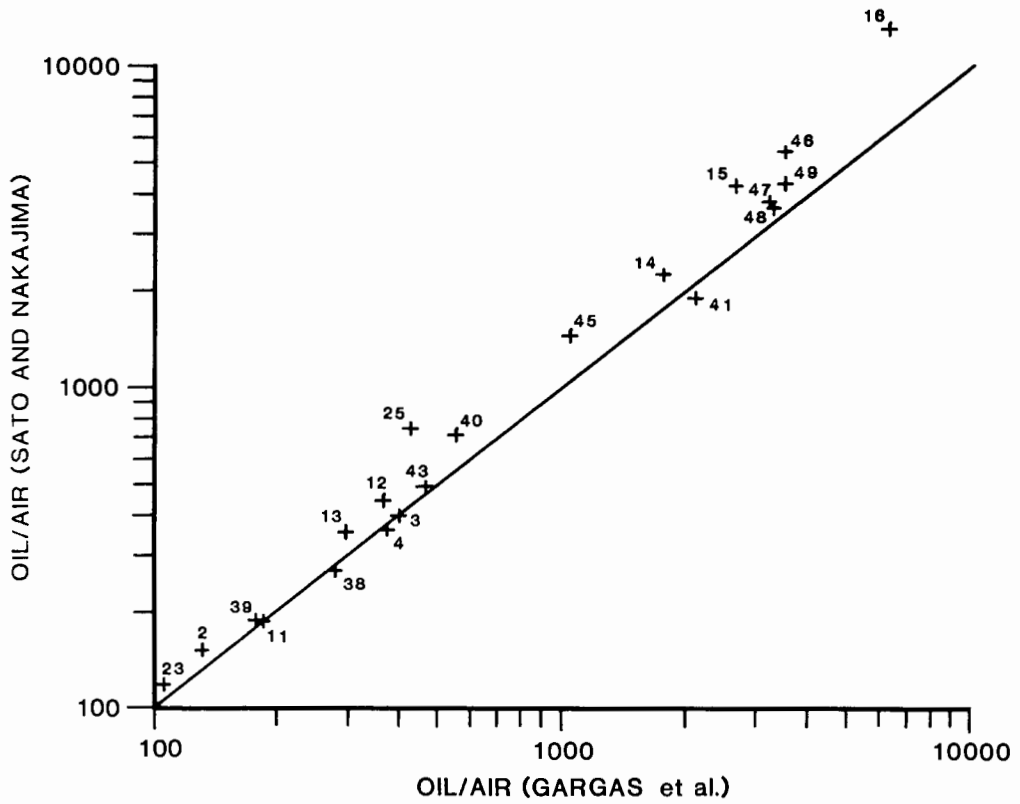

FIG. 1. Comparison of the olive oil:air partition coefficients of Sato and Nakajima (1979a,b) with the results reported here. The solid line has a slope of 1.0 and the numbers identify the individual compounds (see Tables 1–7). The correlation between the two data sets has an r^2 of 0.982.

other human bloods tested. In addition, the individuals that did possess the active process varied in their apparent rates of uptake, which is consistent with the variability in amount of glutathione *S*-transferase present in human erythrocytes between individuals (Scott and Wright, 1980). To ensure that the addition of DEM did not alter partitioning, rat blood was treated with DEM and the partition coefficient for diethyl ether (a compound with no observed continued uptake) was determined. There was essentially no difference between the blood:air partition coefficients determined on DEM-treated blood and those on nontreated blood.

Ethyl acetate and vinyl acetate were found to be readily metabolized by rat blood and human blood, with uptake from the vial headspace continuing well beyond 3 hr. A blood esterase is most probably involved but no definitive evidence is yet available. Blood:air partition coefficient measurements for this chemical in rat and human will not be possible until an effective inhibitor can be found, and no other partition coefficients have been attempted as of this time.

The preceding examples reinforce the need for determining partition coefficients at a minimum of two time points to detect metabolic or other active processes. It is possible that a reaction may proceed to completion during the first hour of equilibration, in which case no continual uptake would be observed and the presence of an active process could be overlooked. An indication of this would be an unusually large value for the partition coefficient or the absence of any measurable chemical concentration in the headspace. The potential of continued uptake from vials containing tissue homogenates, especially liver, also should not be overlooked. The data collected from tissue homogenates

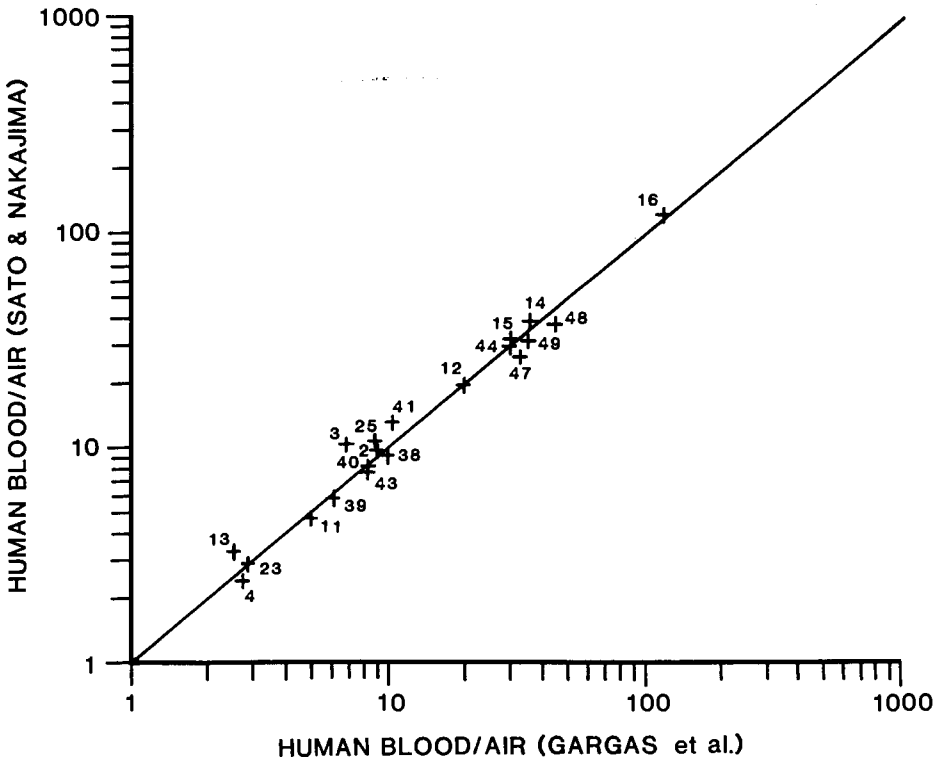

FIG. 2. Comparison of the human blood:air coefficients of Sato and Nakajima (1979a,b) with the results reported here ($r^2 = 0.979$). The numbers correspond to the compound numbers from Table 8 and the solid line has a slope of 1.0.

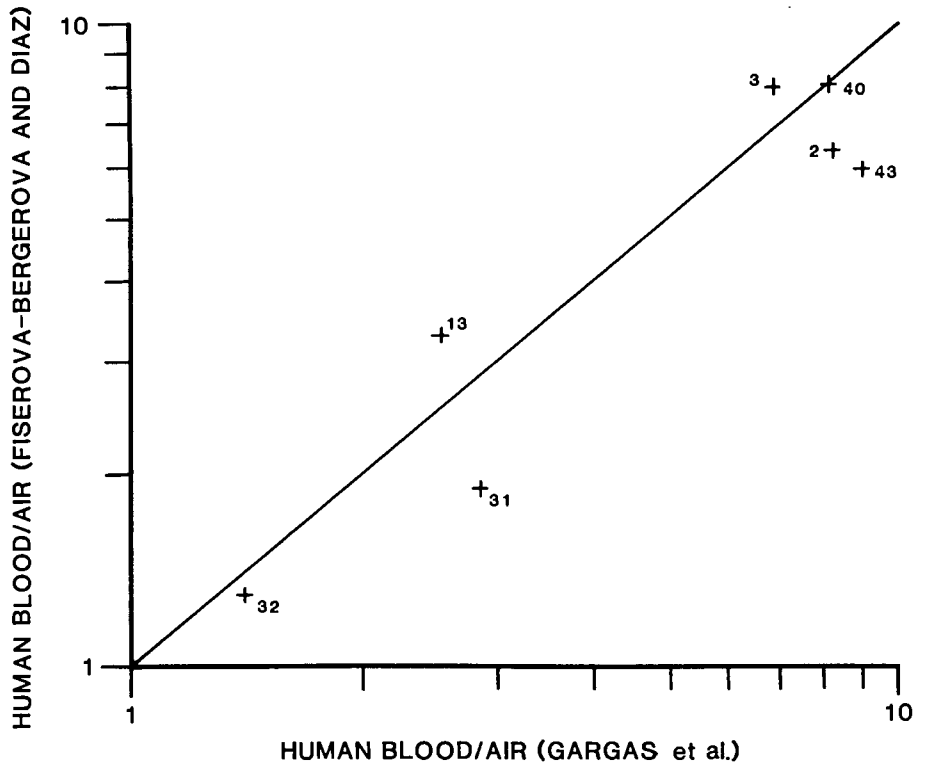

FIG. 3. Limited comparison for seven compounds between the human blood:air coefficients of Fiserova-Bergerova and Diaz (1986) and the present work ($r^2 = 0.876$). The compound numbers are those from Table 8 and the solid line has a slope of 1.0.

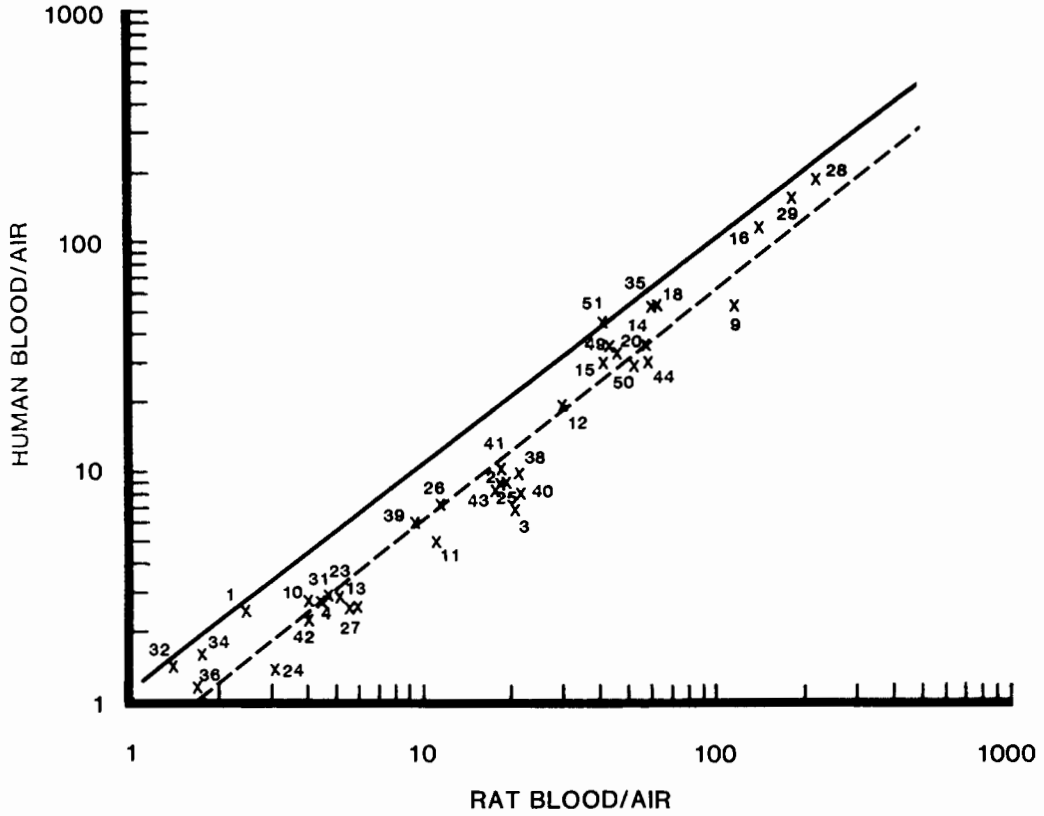

FIG. 4. Comparison of rat blood:air coefficients and human blood:air coefficients for 36 compounds (Table 8). The solid line has a slope of 1.0, and the dashed line is that predicted by Eq. (4) using regression analysis ($r^2 = 0.957$).

in this work were carefully evaluated for continued uptake during the two equilibration time periods. It was not uncommon to reexamine partition coefficients at incubation times of 0.5 and 1.5 hr to ensure equilibrium conditions. For the chemicals reported here, there was no evidence of active metabolic processes in any of the tissues tested, except as noted above for blood. No special treatments of tissue homogenates were performed to ensure enzyme inactivation. This was unnecessary due to the great care needed to maintain an active enzyme (or microsomal) preparation. For example, Sato and Nakajima (1979c) used a vial technique to assess metabolic activity of volatile hydrocarbons. They used a 10,000*g* liver fraction for which great care had to be exercised in chilling the preparation, adjusting the pH of buffers, adding appropriate cofactors (i.e., NADP, $MgCl_2$, glucose 6-phosphate) and in determining optimum conditions and concentrations for observation of metabolic activity. Any sustained metabolic activity was unlikely in our homogenates since no cofactor-generating system was added to our preparation. In addition, Thomas (1975) and Fiserova-Bergerova *et al.* (1984) measured partition coefficients on a variety of tissues, including liver, homogenized in saline for groups of halogenated compounds and reported no evidence of metabolic activity. No special inactivation of enzyme activity was necessary in those studies, nor in these current investigations. As described above, at least two time points were used to ensure equilibrium and were considered adequate for identifying active processes.

Rat fat:air partition coefficients for compounds can be determined from the corresponding oil:air coefficients by applying Eq. (3). Similarly, human blood:air coefficients

can be estimated based on rat blood:air values [Eq. (4)]. As a caution, these equations should be used only for compounds with structures similar to those used to derive these equations. It is possible that the relationships may not hold for more complex molecules. These same cautions apply to estimations of human blood and rat tissue chemical solubility using Eqs. (5)–(9) and the corresponding oil:air and saline:air coefficients. The use of these equations is attractive, but we would suggest that they be used for estimating chemical solubility only as a preliminary method prior to actual experimental determinations.

The coefficients of $\log(P_{oil})$ and $\log(P_{sal})$ in Eqs. (5)–(9) may represent, in a simplistic way, the relative lipophilicity and hydrophilicity of the various tissues. The relative ranking of increasing lipid character and decreasing aqueous character based on these coefficients is $P_{blood} \cong P_{HBL} < P_{muscle} < P_{liver} < P_{fat}$. In fact, the coefficient of $\log(P_{sal})$ for P_{fat} enters the equation with a negative sign [Eq. (8)], indicating very little, if any, aqueous component present in that tissue. The equations from this modeling approach may provide additional insights into the general content (lipid and aqueous) of these various tissues which could otherwise be only assumed or experimentally determined.

The techniques presented here offer a method for determining liquid:air and tissue:air partition coefficients for volatile chemicals. Results from this modified technique compared very well with those for the two methods from which it was developed. Possible means of estimating blood and other tissue solubilities for similar compounds were presented based on oil:air and saline:air contributions and regression analyses. Partition coefficients for a number of these chemicals have already been successfully used in a variety of PB-PK models (McDougal *et al.*, 1986; Gargas *et al.*, 1986a,b; Andersen *et al.*, 1987a,b; D'Souza *et al.*, 1988). The results for the remaining chemicals can now be incorporated into other PB-PK models for use in chemical diposition studies and in assessment of the risk posed by exposure to these chemicals.

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