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Groups of 4 male Wistar rats were dosed intravenously with ^{14}C-labeled benzo[a]pyrene dissolved in an Emulphor/water vehicle at 3 different dose levels and killed at 1 of 15 specific time intervals from 5 min to 32 h after dosing. ^{14}C-Radiolabel concentration–time data were obtained for blood, brain, adipose, heart, kidney, liver, lung, spleen, and testes. Benzo[a]pyrene concentration–time data were obtained for blood, adipose, kidney, liver, and lung. Appropriate mathematical models were fitted to these data and to the data for metabolites derived as the residuals from ^{14}C-radiolabel minus benzo[a]-pyrene difference, where applicable. Nonlinear kinetics were found for ^{14}C-radiolabel in liver, while the data from lung for both ^{14}C-radiolabel and for benzo[a]pyrene per se supported the binding of benzo[a]pyrene in that tissue.

Benzo[a]pyrene (BaP) has been, and remains, one of the most studied chemicals, due largely to its dramatic and wide-ranging toxic effects in mammalian systems (Collins et al., 1991; Forster, 1988; IARC, 1983), and the ubiquitous nature of its occurrence. In spite of the extent of the research involving this chemical, there remain gaps in understanding the pharmacokinetics of benzo[a]pyrene. In 1959, Kotin et al. reported vehicular effects in the elimination of BaP when given subcutaneously or intratracheally, but minimal effects when given intravenously to rats, as all radioactivity was cleared from blood in less than 10 min when injected as a plasma solution of 55 μg/0.15 μCi/kg. Schlede et al. (1970) were able to follow the time course of ^{3}H-BaP concentration in blood for 6 h in their study on the effect of pretreatment of animals with various polycyclic aromatic hydrocarbons (PAHs). The dose used in their study was 50 μg/kg (250 μCi)/kg, and the high specific activity permitted the determination of blood concentrations over a longer period of time. Blood was sampled at 1, 2, 5, 15, and 45 min and 2, 4, and 6 h, and concentration–time information was reported for fat, brain and liver. No attempt was made to mathematically describe the kinetics of the elimination of radioactivity from blood. Both of the studies just described followed the time course of radioactivity derived from the administered dose of BaP. However, it would have been more informative to provide data on the composition of

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that radioactivity, at least as to whether it was parent BaP or metabolite. Wiersma and Roth (1983) described the kinetics of removal of BaP itself from the blood of rats over a 5-h period after administration by injection via several routes and after pretreatment with methylcholanthrene when dosed at about 30 μg/kg. Withey et al. (1993) studied the kinetics following the inhalation of BaP in pregnant rats.

In the present study, we investigated the BaP pharmacokinetics in rats killed at precise times postdosing, with their blood and tissues analyzed for "free" BaP and ^{14}C activity. This allowed an examination of the pharmacokinetics and disposition in blood and tissues of interest.

METHODS

Male Wistar rats weighing approximately 250 g were purchased from Charles River Canada, Inc. (St. Constant, Quebec) and were allowed to acclimatize for 1 wk before dosing. The animals were housed individually and were given rat chow (Lab Chow 5002, PMI Feed) and water ad libitum. Lighting was maintained on a 12-h light/dark cycle while the room temperature and relative humidity were kept at 21 ± 3°C and 30–70%, respectively. At dosing, the animals weighed an average of 324 ± 39 g. The dosing solutions were prepared as follows: For 2 mg/kg, 3.4 mg of ^{14}C-labeled BaP (stated specific activity of 22.3 mCi/mmol) was mixed with 56.7 mg of unlabeled BaP and dissolved in 6 ml of Emulphor (Domtar EL719); 24 ml of 0.9% saline was added so that the final solution was 2 mg/ml/kg body weight/5 μCi. The 6- and 15-mg/kg dosing solutions were prepared in an analogous fashion to provide solutions of 6 mg/3 ml/kg body weight/5.4 μCi and 15 mg/7.5 ml/kg body weight/6 μCi, respectively. Animals were weighed and then lightly anesthetized with isoflurane and injected, via the penile vein, with the desired individual dose administered by adjusting the volume of injection to provide 2, 6, or 15 mg/kg body weight. Urine and feces were collected from the animals that were killed at 8, 24, or 32 h. Animals were killed by cervical dislocation in groups of 4 at 1 of 15 specific times postdosing, ranging from 5 min to 32 h. Careful scheduling allowed all 60 animals in each dose group to be dosed and killed within 3 d. Blood was removed by cardiac puncture, mixed with heparin, and stored in dry ice. Tissues were removed and weighed and a piece for analysis was removed and weighed at the same time. All tissues were immediately placed on dry ice and frozen until analyzed for total ^{14}C-equivalents by scintillation counting or for BaP content by high-performance liquid chromatography (HPLC) analysis with fluorescence detection (Withey et al., 1993).

^{14}C ANALYSIS

Blood, liver, fat, kidney, heart, lung, spleen, brain, testes, urine, and feces were all assayed for total ^{14}C activity. Counting efficiencies ranged from 85 to 95% and were optimized by adjustment of the counting window

by the application of an automatic quench compenstation routine. Portions of the urine (1 ml) were mixed with 12 ml of Hionic Fluor and counted directly (Beckman LS5000TD). One hundred to 200 mg of accurately weighed fecal material was placed into a scintillation vial, 0.2 ml of 60% perchloric acid was added followed by 0.4 ml of 30% hydrogen peroxide, and the mixture was incubated at 60°C with shaking until the material dissolved. Ten milliliters of Biofluor was added and the mixture was allowed to stand in the dark for 1 h before counting. Other wet tissues, except adipose, were homogenized as described later and duplicate 1-ml aliquots of the homogenate were digested with 1 ml Soluene-350 (Packard) at 50°C for 2 h and, if necessary, decolorized with 0.2 ml of 30% hydrogen peroxide. The digest was mixed with 12 ml of Hionic Fluor and counted for radioactivity. Aliquots from the homogenate of a 1-g sample contained about 160 mg of tissue. An accurately known mass of adipose tissue of about 100 mg was digested in 1 ml of Soluene-350 at 50°C for 2 h, mixed with Hionic Fluor, and counted. Portions of blood (0.2 ml) were dissolved in 1.5 ml of Soluene-350:isopropanol (1:1), decolorized with 0.5 ml 30% hydrogen peroxide, and treated in the same fashion as the tissue samples.

EXTRACTION OF BaP FROM NONADIPOSE TISSUE

The extraction method was similar to that of Withey et al. (1993). An accurately weighed portion of about 1 g of whole blood or tissue was homogenized with 5 ml of 0.9% sodium chloride solution. The homogenate was vortexed for about 30 s immediately before removal of 2 ml to a scintillation vial, and 2 ml of 1 *N* sulfuric acid was added. The mixture was extracted for 30 min (Multi-Wrist Shaker, Lab-Line Instruments, Inc., Melrose Park, IL) with 4 ml dichloromethane containing 0.4 μg of 1,2-benzanthracene (BA) (100 ng/ml) as internal standard, then centrifuged for 20 min at 1500 × g (MSE Centaur 2, Johns Scientific, Toronto, Canada). Two milliliters of the lower organic layer was applied to a small, disposable Florisil column (Chromosep FL SPE 500 mg/2.8 ml, Chromatographic Specialties, Brockville, Ontario) attached to a Millex-HV filter (Millipore, Cambridge, MA). Each column was further eluted twice with 2 ml dichloromethane and the combined eluents were evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 2 ml acetonitrile for analysis by HPLC.

EXTRACTION METHOD FOR BaP IN ADIPOSE TISSUE

An accurately weighed portion (300–500 mg) of adipose tissue was homogenized with 5 ml of 0.9% saline. The homogenate was vortexed immediately before removal of 2 ml, which was then extracted for 30 min with 4 ml hexane containing 0.4 μg BA, then centrifuged as already described. The adipose extraction, which employed dimethyl sulfoxide (DMSO), relied on the differential solubility of lipids and BaP in hexane

and DMSO (Haenni et al., 1962). Exactly 2 ml of the hexane layer was removed and extracted with 4 ml DMSO by shaking for 20 min, and the mixture was centrifuged at 1500 × g for 20 min. A portion (1 ml) of the lower DMSO layer was dissolved in 2 ml acetonitrile for analysis by HPLC.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The HPLC consisted of two Waters 510 pumps, a WISP 712 autosampler, and a Shimadzu RF 535 fluorescence detector with the excitation wavelength set at 260 nm and emission wavelength of 406 nm. These were interfaced through a Waters system interface module to a data station running Waters Baseline software (Waters, Mississauga, Ontario). The column was a Waters NovaPak C-18 (150 × 3.9 mm, 4 μm, 60 Å), protected by a guard column (Waters) of the same packing. The mobile phase was an isocratic mixture of acetonitrile:water (86:14), pumped separately. Each component was filtered and degassed prior to use. The flow rate was 0.95 ml/min. Twenty-microliter aliquots of the extracted sample were injected and analyzed against solution standards containing between 1.5 and 150 ng/ml of BaP and BA. Each sample was analyzed in duplicate, with a run time of 9 min.

Extraction efficiencies for liver and adipose were determined by spiking pieces of tissue with 100 μl of a hexane solution of BaP. After 30 min, the tissues were extracted as described earlier and analyzed against solution standards. The determined concentrations were divided by the concentrations expected if 100% was recovered. From liver, recovery was 98% ± 0.7 (n = 2, mean ± SD) and from adipose the recovery was not as high, being 74.4% ± 5.6 (n = 20, mean ± SD) normalized against the recovery of BA. Interday variability samples were prepared from a solution of BA and BaP at about 40 ng/ml each in acetonitrile; 14 aliquots were placed into separate autosampler vials covered with aluminum foil and refrigerated until analysis on 14 consecutive working days. Each day, a sample was allowed to equilibrate to room temperature and then analyzed against a series of solution standards. The percent relative standard deviations for BA and BaP were 3.93 and 3.24%, respectively. In order to assess intraday variability, a solution containing BA and BaP, at about 40 ng/ml each, was analyzed by duplicate injections against a set of solution standards early in the day and then reanalyzed 7 h later. The percent relative standard deviations for BA and BaP were 0.43% and 0.42%, respectively. The response of the system was seen to be linear over the range from 1.5 to 150 ng/ml, with a typical r^2 value of .999.

STATISTICAL METHODS

For the data from the blood, heart, kidney, liver, lung, and spleen, the following model, involving the sum of two exponentials, was fitted to the scintillation counting and HPLC data for each dose group:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where $C(t)$ is the concentration at time t, A and B are the preexponential coefficients, and α and β are the hybrid rate coefficients.

Model 2, as follows, was fitted to the data for the remaining tissues:

$$C(t) = Ae^{-\alpha(t-t_{lag})} + Be^{-\beta(t-t_{lag})} - (A + B)e^{-k_{abs}(t-t_{lag})} \quad (2)$$

where $C(t)$, t, A, B, α, and β are as in model (1), k_{abs} is an absorption rate coefficient, and t_{lag} is a lag time for the absorption.

Model 1 fitted the data best for the blood, heart, kidney, liver, lung, and spleen. The dose group profile for these tissues showed the highest concentration to be at 5 min (the initial measurement time) and, in general, concentrations decreased monotonically with time. However, for the brain, fat, and testes, the concentration of the radioactivity and of BaP itself did not peak until after the initial measurement and did not begin decreasing until later; accordingly, model 2 was fitted to the data for the brain, fat, and testes.

Metabolite concentrations were calculated as the concentration of total radioactivity expressed as ^{14}C-BaP equivalents minus total BaP concentration. For the metabolite data, model 1 was fitted to the data for the blood, heart, liver and spleen, and model 2 was fitted to the data for the brain, fat, and kidney. As there was too little HPLC data to use for the testes, sufficient metabolite data for the testes was not available to determine the kinetics for this tissue.

The rate coefficients for the two-compartment models were calculated as follows:

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \qquad k_{el} = \frac{\alpha\beta}{k_{21}} \qquad k_{12} = \alpha + \beta - k_{21} - k_{el} \qquad V = \frac{\text{dose}}{A + B}$$

where k_{12} is the coefficient of transfer from compartment 1 to compartment 2, k_{21} is the coefficient of transfer from compartment 2 to compartment 1, k_{el} is the coefficient of elimination from compartment 1, and V is the apparent volume of distribution.

Models 1 and 2 were fitted to the data using the procedure NLIN in SAS (SAS Institute, Inc., Carey, NC, 1990), using $(C_{pred} + K)^{-1}$ as weights, where C_{pred} is the predicted concentration and K was 0.005 for BaP and 0.01 for total ^{14}C and the metabolites. The models were fitted using a procedure known as iterative reweighted least squares (IRLS, SAS Institute, Inc.). For IRLS, the model was fitted using nonlinear least squares with the weights calculated as $[C(t) + K]^{-1}$ for the first iteration and recalculated as $(C_{pred} + K)^{-1}$ for each subsequent iteration until the model converged. Asymptotic standard errors were calculated for the estimates of A, B, α, and β, and k_{abs} and t_{lag} for model 2 only, by NLIN.

RESULTS

Table 1 shows the percent of dose excreted in urine and feces at 480, 1440, and 1920 min. At 1920 min (32 h), animals in the 2 mg/kg dose level had excreted 26% of the dose in the feces and about 6% in the urine. At the 2 higher dose levels, fecal excretion was greater than 50%, while urinary excretion remained consistent at 6% for the 6-mg/kg dose group and 7% for the 15-mg/kg group. Tables 2, 3, and 4 show the concentrations in μg ^{14}C-equivalents of BaP per gram fresh weight of tissue, as determined by scintillation counting, for blood, brain, adipose, heart, kidney, liver, lung, spleen, and testes for the dose levels 2, 6, and 15 mg/kg respectively. Tables 5, 6, and 7 show parent BaP concentrations in blood, adipose, kidney, liver, and lung as determined by HPLC analysis for the various dose levels. Tables 8 and 9 present kinetic parameter estimates for ^{14}C observations and BaP observations, respectively, and Table 10 gives analogous estimates for metabolites. The metabolite concentrations were estimated as the residuals from the ^{14}C and BaP measurements. Tables 11, 12, and 13 provide estimates of rate coefficients, apparent volumes of distribution, and half-lives for ^{14}C equivalents, BaP, and metabolites, respectively.

For radiolabel in blood, the 6-mg/kg dose group showed a concentration increase at 8 h (480 min) that was not seen at the other two dose levels (Figure 1 and Table 3). For BaP itself, in both the 2- and 6-mg/kg groups, values rapidly declined below the limit of detection (Figure 2) and the data were very similar, with β-phase half-lives of 36 and 25 min, respectively (Table 12). These declines could easily be described by a monoexponential equation, but for consistency with other tissues and doses, and somewhat arbitrarily, these phenomena have also been described biexponentially. For the 15-mg/kg dose group, BaP was also

TABLE 1. Excretion of radioactive materials in urine and feces of rats administered BaP intravenously

Dose (mg/kg)	Time (min)	Urine (% of dose)	Feces (% of dose)
2	480	4.27 ± 0.44	0.06 ± 0.3(3)
2	1440	6.10 ± 1.01	38.7 ± 19.0
2	1920	6.15 ± 0.60(3)	26.1 ± 12.7
6	480	2.56 ± 0.82	5.55 ± 6.3
6	1440	6.67 ± 3.16(2)	42.9 ± 12.2(2)
6	1920	5.65 ± 1.09	56.0 ± 11.4
15	480	3.16 ± 0.51	0.43 ± 0.73
15	1440	6.53 ± 1.12	41.4 ± 4.4
15	1920	7.23 ± 0.28	50.1 ± 7.9

Note. Unless otherwise given in parentheses, results denote means ± SD obtained from groups of four animals.

TABLE 2. Tissue distribution of radioactivity in rats administered intravenously ^{14}C-BaP at 2 mg/kg body weight

	Time (min)							
Tissue	5[a]	10[a]	20	30	45	60	90	120[a]
Blood	4.27 ± 0.25	2.6 ± 0.32	2.22 ± 0.25	1.3 ± 0.13	0.95 ± 0.06	0.94 ± 0.12	0.85 ± 0.08	0.81 ± 0.09
Brain	1.77 ± 0.17	1.69 ± 0.57	2.42 ± 0.41	1.92 ± 0.07	1.98 ± 0.08	2.07 ± 0.14	1.88 ± 0.18	1.73 ± 0.28
Adipose	0.82 ± 0.58	1.03 ± 0.45	1.48 ± 0.11	1.78 ± 0.78	1.3 ± 0.18	2.02 ± 0.51	1.76 ± 0.30	2.31 ± 0.87
Heart	10.81 ± 1.34	6.02 ± 1.24	6.00 ± 2.03	2.3 ± 0.40	1.29 ± 0.23	1.17 ± 0.05	0.68 ± 0.15	0.44 ± 0.07
Kidney	8.94 ± 1.21	6.02 ± 0.25	8.87 ± 1.40	4.72 ± 0.25	3.99 ± 0.28	4.16 ± 0.26	5.36 ± 2.09	4.58 ± 0.80
Liver	20.55 ± 2.20	17.19 ± 1.92	11.52 ± 1.71	8.78 ± 0.68	6.98 ± 0.47	6.68 ± 0.59	4.56 ± 0.51	0.24 ± 0.05
Lung	40.54 ± 4.85	18.05 ± 5.26	8.4 ± 2.55	5.87 ± 2.52	3.35 ± 0.45	3.09 ± 0.40	2.06 ± 0.29	11.15 ± 6.90
Spleen	5.78 ± 1.09	3.45 ± 0.58	3.64 ± 0.64	1.9 ± 0.20	0.96 ± 0.19	0.8 ± 0.12	0.54 ± 0.10	0.36 ± 0.05
Testes	0.4 ± 0.06	0.81 ± 0.12	0.3 ± 0.07	0.52 ± 0.02	0.48 ± 0.02	0.47 ± 0.05	0.5 ± 0.07	0.42 ± 0.09

	Time (min)						
Tissue	180[a]	240	300	360	480	1440	1920
Blood	0.65 ± 0.15	0.54 ± 0.07	0.47 ± 0.16	0.43 ± 0.10	0.39 ± 0.06	0.13 ± 0.01	0.11 ± 0.05
Brain	1.34 ± 0.06	1.04 ± 0.19	0.77 ± 0.06	0.63 ± 0.11	0.45 ± 0.08	0.09 ± 0.02	0.05 ± 0.00
Adipose	1.99 ± 0.19	1.83 ± 0.55	1.83 ± 0.70	1.81 ± 0.45	1.76 ± 0.34	0.49 ± 0.33	0.78 ± 0.49
Heart	0.33 ± 0.02	0.25 ± 0.05	0.21 ± 0.03	0.25 ± 0.02	0.19 ± 0.03	0.08 ± 0.01	0 07 ± 0.02
Kidney	4.86 ± 0.49	4.62 ± 0.53	4.6 ± 0.79	4.36 ± 0.56	3.74 ± 0.57	1.2 ± 0.33	0.98 ± 0.23
Liver	0.22 ± 0.03	0.16 ± 0.03	0.11 ± 0.03	0.11 ± 0.06	0.07 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Lung	1.12 ± 0.21	0.64 ± 0.19	0.9 ± 0.57	0.58 ± 0.08	5.55 ± 3.23	0.16 ± 0.03	0.13 ± 0.04
Spleen	0.31 ± 0.02	0.22 ± 0.02	0.2 ± 0.03	0.17 ± 0.01	0.14 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Testes	0.33 ± 0.06	0.26 ± 0.03	0.23 ± 0.03	0.2 ± 0.03	0.15 ± 0.04	0.05 ± 0.01	0.05 ± 0.02

Note. Results represent µg/g tissue of ^{14}C-equivalents, expressed as means ± SD of four rats.
[a]$n = 3$.

TABLE 3. Tissue distribution of radioactivity in rats administered ^{14}C-BaP intravenously at 6 mg/kg

	Time (min)							
Tissue	5	10[a]	20	30[a]	45[b]	60[b]	90	120
Blood	12.78 ± 1.79	11.55 ± 1.51	10.00 ± 0.92	5.77 ± 1.01	4.91 ± 0.62	6.01 ± 1.95	3.42 ± 0.34	2.88 ± 0.44
Brain	3.43 ± 0.77	4.43 ± 0.13	5.88 ± 0.26	6.04 ± 0.24	7.42 ± 1.93	7.86 ± 0.18	6.27 ± 0.29	6.61 ± 0.49
Adipose	1.83 ± 0.25	2.74 ± 0.55	4.16 ± 0.41	4.97 ± 0.23	1.78 ± 0.94	1.67 ± 0.77	9.20 ± 0.92	9.54 ± 0.74
Heart	17.79 ± 2.50	14.48 ± 1.67	10.99 ± 0.84	8.19 ± 0.96	6.32 ± 0.54	5.00 ± 0.07	3.20 ± 0.84	2.10 ± 0.02
Kidney	15.61 ± 1.51	16.51 ± 0.23	14.98 ± 0.68	13.68 ± 0.50	11.93 ± 0.89	10.49 ± 1.65	9.72 ± 0.57	10.83 ± 1.52
Liver	44.70 ± 5.73	37.74 ± 3.24	28.42 ± 0.39	21.58 ± 1.10	16.85 ± 0.52	17.88 ± 1.62	11.20 ± 0.88	9.47 ± 0.80
Lung	38.83 ± 8.18	19.73 ± 3.33	23.55 ± 7.28	26.06 ± 8.93	9.64 ± 0.80	8.46 ± 0.22	8.48 ± 1.48	12.43 ± 1.13
Spleen	5.09 ± 0.87	2.80 ± 1.13	9.03 ± 0.52	7.10 ± 0.49	5.13 ± 0.21	5.76 ± 1.97	2.31 ± 0.37	1.75 ± 0.27
Testes	1.08 ± 0.38	1.26 ± 0.07	1.35 ± 0.14	1.34 ± 0.15	1.40 ± 0.08	1.87 ± 0.24	1.68 ± 0.06	1.53 ± 0.22

	Time (min)						
Tissue	180[a]	240[a]	300	360	480[a]	1440[b]	1920
Blood	2.04 ± 0.23	2.38 ± 0.56	2.13 ± 0.27	2.01 ± 0.40	2.87 ± 1.18	0.84 ± 0.10	0.67 ± 0.02
Brain	4.94 ± 2.08	3.29 ± 1.13	2.48 ± 0.53	2.43 ± 0.82	1.36 ± 0.27	0.39 ± 0.04	0.11 ± 0.05
Adipose	6.74 ± 0.75	6.75 ± 3.25	8.13 ± 1.06	8.10 ± 0.49	8.67 ± 0.99	0.56 ± 0.00	3.14 ± 0.66
Heart	1.04 ± 0.19	1.84 ± 0.53	1.15 ± 0.55	0.90 ± 0.14	0.62 ± 0.10	2.19 ± 0.11	0.11 ± 0.01
Kidney	13.27 ± 2.86	13.42 ± 2.32	12.42 ± 1.18	12.52 ± 1.48	10.69 ± 1.34	3.32 ± 0.14	1.93 ± 0.31
Liver	6.25 ± 0.61	5.00 ± 0.72	3.33 ± 0.22	3.38 ± 0.59	3.04 ± 0.58	0.85 ± 0.14	0.66 ± 0.23
Lung	2.64 ± 0.83	5.82 ± 3.20	14.27 ± 2.32	8.08 ± 4.08	6.27 ± 0.70	0.93 ± 0.06	4.48 ± 1.77
Spleen	0.92 ± 0.34	0.93 ± 0.35	0.75 ± 0.10	0.56 ± 0.16	0.64 ± 0.11	0.35 ± 0.02	0.21 ± 0.05
Testes	1.00 ± 0.21	0.76 ± 0.30	0.69 ± 0.18	0.56 ± 0.22	0.44 ± 0.18	0.11 ± 0.01	0.07 ± 0.01

Note. Unless indicated otherwise, data denote μg/g tissue of ^{14}C-equivalents, expressed as means ± SD obtained from four rats.
[a] $n = 3$.
[b] $n = 2$.

TABLE 4. Tissue distribution of radioactivity in rats administered ^{14}C-BaP intravenously at 15 mg/kg

	Time (min)							
Tissue	5	10	20	30	45	60	90	120
Blood	35.8 ± 4	32.3 ± 2.91	25.5 ± 1.05	22.2 ± 1.27	17.8 ± 0.75	17.5 ± 1.33	15.1 ± 2.58	12.4 ± 1.12
Brain	9.32 ± 0.96	11.8 ± 0.88	14.5 ± 1.66	16.9 ± 0.66	18.6 ± 1.9	21.3 ± 1.52	22.9 ± 3.56	20.1 ± 2.78
Adipose	2.32 ± 0.43	6.02 ± 1.78	9.45 ± 1.82	15.1 ± 1.68	20 ± 6.45	18.5 ± 4.35	19.9 ± 9.77	28.6 ± 3.26
Heart	45.5 ± 3.81	40.1 ± 0.98	30.4 ± 1.88	23.3 ± 1.72	19.8 ± 0.8	16.6 ± 1.81	16.2 ± 4.33	8.01 ± 1.75
Kidney	38.8 ± 2.49	39.4 ± 2.11	39.5 ± 1.79	37.4 ± 0.61	34.3 ± 2.12	34.2 ± 2.6	30.4 ± 3.13	26.6 ± 2.1
Liver	108.9 ± 5.58	94.38 ± 8.77	70.48 ± 7.49	58.09 ± 1.73	55.81 ± 1.00	45.1 ± 7.31	40.17 ± 10.20	21.95 ± 7.19
Lung	70.44 ± 18.22	40.21 ± 5.62	34.16 ± 4.42	43.17 ± 9.19	26.71 ± 8.24	27.27 ± 16.63	22.86 ± 5.14	19.26 ± 11.93
Spleen	3.52 ± 3.78	7.41 ± 5.73	4.99 ± 1.22	3.28 ± 1.72	8.08 ± 0.79	5.29 ± 1.44	4.28 ± 5.29	7.54 ± 1.34
Testes	2.48 ± 0.50	3.21 ± 0.43	3.56 ± 0.60	4.08 ± 0.23	4.81 ± 0.46	5.26 ± 0.24	5.3 ± 0.69	4.73 ± 0.33

	Time (min)						
Tissue	180	240	300	360	480	1440	1920
Blood	9.1 ± 1.47	6.02 ± 1.22	5.18 ± 0.53	4.01 ± 0.44	4.15 ± 0.69	1.6 ± 0.2	0.89 ± 0.11
Brain	18.3 ± 3.24	14.5 ± 5.45	10.5 ± 2.46	7.2 ± 0.74	4.91 ± 0.83	0.64 ± 0.06	0.31 ± 0.05
Adipose	36.3 ± 10.8	29.9 ± 6.03	39.6 ± 8.99	26.8 ± 6.69	28.7 ± 4.38	20.1 ± 1.87	13.8 ± 1.82
Heart	5.59 ± 1.54	3.28 ± 1.02	2.39 ± 0.33	1.84 ± 0.07	2.02 ± 0.1	1.23 ± 0.28	0.5 ± 0.05
Kidney	30.9 ± 3.49	35.9 ± 2.2	31.1 ± 3.2	33.6 ± 4.47	34.2 ± 2.81	11.4 ± 2.21	5.91 ± 0.84
Liver	23.05 ± 3.41	17.54 ± 3.21	12.6 ± 2.04	8.79 ± 0.56	7.8 ± 0.66	2.81 ± 0.54	1.65 ± 0.29
Lung	9.18 ± 0.95	12.71 ± 3.80	13.53 ± 8.05	12.37 ± 1.64	14.35 ± 13.11	5.91 ± 1.78	9. ± 1.30
Spleen	4.32 ± 0.80	3.1 ± 1.0	2.8 ± 0.38	2.28 ± 0.22	2.75 ± 0.45	0.98 ± 0.11	0.62 ± 0.10
Testes	4.69 ± 0.73	3.83 ± 1.22	3.03 ± 0.52	1.72 ± 0.09	1.76 ± 0.10	0.39 ± 0.06	0.21 ± 0.04

Note. Results are mean concentrations (μg/g tissue) ± SD expressed as ^{14}C-equivalents obtained from groups of four animals.

TABLE 5. Distribution of BaP in tissues of rats dosed intravenously with 2 mg/kg of BaP

Time (min)	Tissue				
	Blood	Adipose	Kidney	Liver	Lung
5[a]	2.64 ± 0.84	1.06 ± 0.92	7.00 ± 2.69	11.33 ± 4.66	5.17 ± 1.15
10	1.85[a] ± 0.58	1.22 ± 0.29	7.09 ± 0.78	6.52 ± 1.23	1.31 ± 0.26
20	1.2 ± 0.36	1.82 ± 0.62	7.68 ± 1.23	4.01 ± 0.68	2.80 ± 1.84
30	0.66 ± 0.21	2.77 ± 1.23	4.64 ± 0.35	2.43 ± 0.56	1.06 ± 0.45
45	0.47 ± 0.09	2.4 ± 0.55	4.02 ± 0.75	1.34 ± 0.56	2.68 ± 0.99
60	0.42 ± 0.06	3.86 ± 0.68	2.67 ± 0.75	0.34 ± 0.11	2.40 ± 0.41
90	0.17 ± 0.05	2.58 ± 0.48	1.31 ± 0.89	0.91 ± 0.45	1.07 ± 0.48
120	0.35[b] ± 0.23	3.96 ± 1.92	0.21 ± 0.06	0.01 ± 0.003	14.65 ± 1.67
180	ND	3.29 ± 0.68	0.23 ± 0.10	ND	0.34 ± 0.20
240	ND	2.96 ± 1.13	0.07[a] ± 0.02	ND	0.04 ± 0.02
300	ND	3.03 ± 1.13	0.06 ± 0.06	ND	0.68 ± 0.79
360	ND	2.28 ± 0.17	0.08 ± 0.07	ND	0.06 ± 0.06
480	ND	3.13 ± 0.69	0.08 ± 0.10	ND	9.32 ± 0.94
1440	ND	1.38[b] ± 0.71	ND	ND	ND
1920	ND	1.46[a] ± 0.12	ND	ND	ND

Note. Results denote μg/g tissue of BaP, expressed as means ± SD obtained from groups of four animals.

[a] $n = 3$.

[b] $n = 2$.

TABLE 6. Distribution of BaP in tissues of rats dosed intravenously with BaP at 6 mg/kg

Time (min)	Tissue				
	Blood	Adipose	Kidney	Liver	Lung
5	4.66 ± 0.56	0.37 ± 0.30	12.37 ± 2.25	31.16 ± 4.61	33.90 ± 7.74
10[a]	5. ± 0.19	0.81 ± 0.13	15.88 ± 3.50	24.31 ± 5.63	13.63 ± 1.85
20	3.78 ± 0.55	2.04 ± 0.57	11.69 ± 1.49	16.14 ± 0.93	17.28 ± 6.15
30[a]	2.42 ± 0.21	2.29 ± 0.75	7.09 ± 4.29	11.44 ± 1.56	16.01 ± 7.78
45[b]	1.99 ± 0.14	4.54 ± 1.88	7.57 ± 0.17	11.13 ± 5.87	5.85 ± 1.65
60[b]	1.72 ± 0.29	3.99 ± 0.31	6.12 ± 0.07	5.54 ± 1.45	2.76 ± 0.96
90	0.5 ± 0.14	5.25 ± 0.76	2.25 ± 0.80	1.32 ± 0.60	5.30 ± 1.14
120	0.14[a] ± 0.11	3.37 ± 1.28	0.8[a] ± 0.25	0.55 ± 0.27	5.84 ± 0.66
180[a]	0.03 ± 0.02	2.88 ± 1.11	0.2 ± 0.10	0.15 ± 0.05	0.40 ± 0.31
240[a]	ND	2.38 ± 1.41	0.15 ± 0.09	0.09 ± 0.04	2.64 ± 1.62
300	0.01[a] ± 0.001	3.88 ± 0.15	0.09 ± 0.03	0.06 ± 0.01	12.40 ± 2.96
360	ND	4.26 ± 0.93	0.11 ± 0.04	0.06 ± 0.04	6.27 ± 4.07
480	ND	4.09 ± 0.50	0.09 ± 0.03	0.02 ± 0.003	5.09 ± 1.70
1440	ND	ND	<0.01[b] ± 0.001	0.01[b] ± 0.003	ND
1920	ND	1.02 ± 0.34	<0.01 ± 0.002	0.02[c] ± –	3.90 ± 2.06

Note. Data denote mean concentrations (μg/g tissue) ± SD of four animals.

[a] $n = 3$

[b] $n = 2$.

[c] $n = 1$.

TABLE 7. Distribution of BaP in tissues of rats dosed with the test substance intravenously at 15 mg/kg

Time (min)	Tissue				
	Blood	Adipose	Kidney	Liver	Lung
5	28.83 ± 3.34	1.47 ± 0.48	30.77 ± 2.31	27.5 ± 20.70	45.97 ± 24.12
10	29.82 ± 3.44	2.73 ± 0.77	25.2 ± 2.00	29.82 ± 5.83	26.05 ± 6.57
20	15.89 ± 0.65	5.65 ± 1.40	22.47 ± 1.61	21.93 ± 7.35	23.35 ± 6.03
30	13.78 ± 0.72	8.6 ± 5.15	20.08 ± 1.39	15.25 ± 4.74	25.78 ± 9.83
45	10.57 ± 0.94	13.38 ± 5.77	16.81 ± 0.57	13.08 ± 4.53	13.74 ± 8.04
60	8.52 ± 1.04	9.76 ± 3.31	15.13 ± 2.46	4.86 ± 2.71	16.99 ± 13.39
90	7.16 ± 2.95	14.79 ± 7.93	13.61 ± 5.16	8.51 ± 3.56	38.55 ± 3.02
120	3.93 ± 1.36	17.12 ± 2.76	5.37 ± 1.76	3.39 ± 1.64	36.96 ± 34.45
180	1.86 ± 1.94	19.77 ± 7.09	1.77 ± 1.57	0.72 ± 0.62	7.07 ± 2.45
240	0.19 ± 0.16	15.42 ± 3.99	0.8 ± 0.53	0.24 ± 0.12	22.42 ± 11.36
300	0.09 ± 0.05	27.76 ± 6.69	0.36 ± 0.11	0.14 ± 0.05	33.22 ± 12.95
360	0 05[a] ± 0.02	15.05 ± 3.91	0.35 ± 0.06	0.16 ± 0.10	22.62 ± 3.75
480	0.02 ± 0.03	17.38 ± 4.26	0.25 ± 0.06	0.05 ± 0.02	18.39 ± 26.74
1440	0.015 ± 0.02	11.1 ± 1.40	0.05 ± 0.02	0.01 ± 0.01	10.16 ± 3.88
1920	0.002 ± 0.002	5.85 ± 0.66	0.01 ± 0.003	<0.01[a] ± 0.003	15.65 ± 2.71

Note. Data represent mean concentrations (μg/g tissue) ± SD obtained from groups of four rats.
[a]$n = 3$.

removed from blood very quickly in the initial phase. For 480 min, the rate of decline was similar to that of the lower dose levels, but measurable levels of BaP were determined at 1440 and 1920 min, giving a terminal-phase half-life of nearly 7 h. Figure 3 shows the ^{14}C-equivalents residue values and curves of the best fit of the derived parameters for the liver, while Figure 4 shows the analogous data and curves for BaP itself. The ^{14}C data displayed what might be a saturable process between the 2- and 6-mg/kg dose levels, with the concentration at 120 min at the 2-mg/kg level declining sharply. This was also seen in the BaP concentration, but 120 min was the last sample time at which concentrations could be measured for the 2-mg/kg dose level. The kidney was characterized by a dose-wide increase in ^{14}C concentration beginning between 60 and 90 min and lasting until 4 h postdosing (Tables 2, 3, and 4). The HPLC analyses of "free" BaP did not show the same pattern for the tissues, but showed a continuous biexponential decline (Tables 5, 6, and 7). Adipose tissue showed distinct uptake phases for both BaP (Tables 5, 6, and 7) and ^{14}C-equivalents (Tables 2, 3, and 4). The BaP concentration values at the 2-mg/kg dose level were somewhat abnormally elevated and were at or near those obtained for the 6-mg/kg dose level. Lung tissue provided concentrations that were not as smoothly declining as some of the other tissues, both for ^{14}C-equivalents and BaP. At 5 min, for the 2-mg/kg dose level, lung had higher concentration of ^{14}C-equivalents than any other tissue, and this was true of BaP itself in both the 6- and 15-mg/kg dose

TABLE 8. Kinetic parameters of elimination of total ^{14}C-equivalents from tissues of rats after iv administration

Tissue[g]	Dose	A (μg/g)	B (μg/g)	α (min^{-1})	β (min^{-1})	k_{abs} (min^{-1})	t_{lag} (min)
Brain	2	3.11(0.82)[h]	0.41(0.24)	0.0054(0.001)	0.0011(3E-4)	0.032(0.018)	31.289(21.89)
	6	26.56(127.2)	3.91(1.27)	0.0118(0.015)	0.0018(2E-4)	0.02(0.024)	11.115(5.992)
	15	94.9(153)	5.96(2.41)	0.0077(0.003)	0.0015(2E-4)	0.013(0.006)	14.097(3.781)
Adipose	2	1.27(24.72)	1.11(24.23)	0.0003(0.0049)	0.0011(0.0169)	0.035(0.027)	11.087(11.24)
	6	8.52(18.26)	1.61(16.98)	0.0006(1.105)	0.0006(5.86)	0.023(0.061)	3.964(1.79)
	15	35.86(0)	6.25(7.58)	0.0005(0.145)	0.0005(0.831)	0.014(0.003)	0.165(0.955)
Testes	2	0.75(0.13)	0.06(0.09)	0.0041(0.003)	0.0001(0.001)	0.042(0.064)	63.438(32.66)
	6	2.78(2.81)	0.67(0.41)	0.0072(0.005)	0.0012(4E-4)	0.024(0.019)	22.357(15.46)
	15	43.51(4077)	2.85(1.11)	0.0096(0.053)	0.0014(2E-4)	0.012(0.057)	19.777(6.253)
Blood	2	4.08(0.57)	0.76(0.04)	0.0577(0.007)	0.0011(1E-4)		
	6[a]	11.56(1.43)	2.87(0.21)	0.0306(0.005)	0.0008(1E-4)		
	15	26.74(1.28)	6.1(0.48)	0.0122(0.001)	0.001(1E-4)		
Heart	2[b]	9.87(0.95)	0.34(0.03)	0.0414(0.003)	0.0009(1E-4)		
	6	16.8(1.69)	1.65(0.15)	0.0267(0.003)	0.0014(1E-4)		
	15	39.77(2.58)	2.66(0.30)	0.0151(0.001)	0.0007(1E-4)		
Kidney	2[c]	5.3(0.487)	4.69(2.14)	0.0009(8E-5)	0.0636(0.046)		
	6[d]	9.65(2.34)	7.84(2.76)	0.0008(2E-4)	0.023(0.011)		
	15[c]	31.28(3.96)	10.6(3.29)	0.0008(8E-5)	0.0197(0.0177)		
Liver	2[e]	22.92(1.92)	0.13(0.03)	0.0243(0.001)	0.0011(3E-4)		
	6	36.03(2.29)	5.01(0.35)	0.0175(0.001)	0.0011(1E-4)		
	15	80.22(5.00)	14.37(1.50)	0.0129(0.001)	0.0011(1E-4)		
Lung	2[f]	30.43(5.02)	1.25(0.14)	0.0517(0.0058)	0.0013(1E-4)		
	6	32.08(10.04)	9.34(1.18)	0.043(0.017)	0.0005(2E-2)		
	15	45.44(9.17)	13.66(1.97)	0.0209(0.006)	0.0003(1E-4)		
Spleen	2	5.35(0.46)	0.26(0.02)	0.0355(0.002)	0.0007(9E-5)		
	6	14.5(1.07)	0.88(0.07)	0.0236(0.002)	0.0007(8E-5)		
	15	37.68(2.51)	3.49(0.30)	0.0177(0.001)	0.0009(8E-5)		

[a]Data from 240–480 min ignored.
[b]Data from 1440 min ignored.
[c]Data from 180–480 min ignored.
[d]Data from 120–480 min ignored.
[e]Data from 120 min ignored.
[f]Data from 120 and 480 min ignored.
[g]Data were fitted to the biexponential equation of $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$ for blood, heart, kidney, liver, lung, and spleen. For the remaining tissues, the equation $C(t) = Ae^{-\alpha(t-t_{lag})} + Be^{-\beta(t-t_{lag})} - (A + B)^{-K_{abs}(t-t_{lag})}$ was used.
[h]Data in parentheses are asymptotic error.

levels. The ^{14}C-equivalents data for both the brain and testes show a distinct uptake phase at all dose levels.

Figure 5 shows the plot of the differences of ^{14}C and BaP concentrations in kidney, representing the metabolite concentration along with curves of the equations using the parameters in Table 10. The rate of decline of the radioactivity in the β-phase of the elimination was unaffected by dose.

TABLE 9. Kinetic parameters of elimination of BaP from tissues of rats and curve-fitting to the equation $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$ for rat tissues

Tissue	Dose	A (μg/g)	B (μg/g)	α (min^{-1})	β (min^{-1})	k_{abs} (min^{-1})[c]	t_{lag} (min)
Adipose	2	3.2(7.7)[d]	1.18(8.41)	0.0005(0.002)	0.0029(0.057)	0.029(0.023)	7.475(8.4)
	6	3.77(2.74)	1.23(3.58)	0.0008(5.73)	0.0007(17.60)	0.031(0.014)	0.461(2.15)
	15	27.05(107.25)	2.56(38.18)	0.0008(0.001)	0.0009(0.369)	0.011(0.02)	1.015(1.9)
Blood	2	2.92(1.66)	1.18(0.4)	0.1183(0.076)	0.0191(0.0038)		
	6	6.39(509.4)	0.221(510)	0.028(17.2)	0.028(497)		
	15	30(3.1)	0.03(0.13)	0.016(0.001)	0.0017(0.0073)		
Kidney	2	11.06(1.51)	0.11(0.05)	0.0274(0.002)	0.0014(0.0011)		
	6	19.75(2.11)	0.16(0.05)	0.0278(0.002)	0.0015(0.0008)		
	15	36.912(2.987)	0.49(0.16)	0.0156(0.001)	0.0018(0.0004)		
Liver	2[a]	13.6(7.33)	4.36(1.35)	0.1308(0.068)	0.0246(0.0036)		
	6	37.31(3.56)	0.124(0.082)	0.0346(0.002)	0.0022(0.0019)		
	15	33.32(4.06)	0.22(0.24)	0.0213(0.002)	0.0025(0.0026)		
Lung	2[b]	5.05(0.62)	0.02(0.05)	0.015(0.002)	0.0004(0.002)		
	6	33.25(13.86)	8.97(1.19)	0.061(0.029)	0.0003(0.0002)		
	15	35.10(942.3)	5.70(944.5)	0.0007(0.012)	0.0001(0.007)		

[a]Data from 120 min ignored.
[b]Data from 240–480 min ignored.
[c]Data from adipose tissue were fitted to the equation $C(t) = Ae^{-\alpha(t-t_{lag})} + Be^{\beta(t-t_{lag})} - (A + B)e^{-K_{abs}(t-t_{lag})}$.
[d]Results in parentheses are asymptotic error.

TABLE 10. Kinetic parameters of elimination of total BaP metabolites from tissues of rats and curve-fitting to the equation $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$

Tissue	Dose	A (μg/g)	B (μg/g)	α (min^{-1})	β (min^{-1})	k_{abs} (min^{-1})[c]	t_{lag} (min)
Adipose	6	5.37(4.58)	0.51(2.68)	0.0004(6E-4)	0.0056(0.05)	0.019(0.015)	10.22(6.65)
	15	14.21(561)	1.27(554)	0.0003(0.0009)	0.0009(0.2)	0.019(0.009)	0.001(1.543)
Kidney	2	5.35(3.25)	0.07(4.25)	0.0009(0.002)	0.0001(0.001)	0.029(0.038)	11.56(17.66)
	6	17.29(130)	1.36(112)	0.001(0.003)	0.0029(0.32)	0.01(0.016)	20.34(14.75)
	15	32.63(197)	16.09(267)	0.001(17.1)	0.001(34.7)	0.008(0.009)	30.24(9.84)
Blood	2	1.59(0.56)	0.72(0.05)	0.090(0.03)	0.001(1E-4)		
	6	6.16(1.49)	3.17(0.2)	0.055(0.02)	0.0008(1E-4)		
	15	5.99(5.15)	3.4(4.92)	0.001(5E-4)	0.0039(0.01)		
Heart	2	0.61(0.28)	0.34(0.3)	0.0074(0.008)	0.0012(7E-4)		
Liver	2[a]	14.68(1.24)	0.12(0.03)	0.193(0.001)	0.0011(4E-4)		
	6	11.57(1.19)	3.7(0)	0.0068(0.002)	0.001(3E-4)		
	15	52.03(4)	15.2(1.94)	0.0115(0.002)	0.0012(1E-4)		
Lung	2[b]	53.36(12.62)	0.76(0.09)	0.105(0.012)	0.001(1E-4)		
	6	6.73(1.19)	1.04(1.36)	0.004(0.002)	0.0001(8E-4)		
	15	33.08(18.63)	1.59(0.86)	0.0036(0.016)	0.0024(0.001)		

Note. See footnotes *c* and *d* of Table 9.
[a]Data from 120 min ignored.
[b]Data from 120 and 480 min ignored.

TABLE 11. Rate coefficients, apparent volume of distribution (A.V.D.), and half-lives of total ^{14}C-equivalents in tissues of rats dosed with ^{14}C-BaP intravenously

Tissue	Dose (mg/kg)	k_{12}[g] (min^{-1})	k_{21} (min^{-1})	k_{el} (min^{-1})	A.V.D. (L)	$t_{1/2}$ (min)
Blood	2	0.0423	0.01	0.0065	0.4134	614
	6[a]	0.0212	0.0067	0.0035	0.4159	910
	15	0.0062	0.0031	0.0039	0.4567	709
Brain	2	0.0012	0.0016	0.0036	0.5681	660
	6	0.0037	0.003	0.0068	0.1969	393
	15	0.0011	0.0019	0.0062	0.1487	452
Adipose	2	0.0006	0.0016	0.0005	0.7715	645
	6	0.013	0.0206	0.001	0.3432	1190
	15	0.0023	0.0125	0.0009	0.2427	1302
Heart	2[b]	0.0232	0.0023	0.0168	0.1958	750
	6	0.0141	0.0037	0.0103	0.3252	484
	15	0.0074	0.0016	0.0068	0.3535	944
Kidney	2[c]	0.0287	0.0342	0.0017	0.2003	11
	6[d]	0.0093	0.013	0.0014	0.343	30
	15[c]	0.0045	0.0149	0.001	0.3582	35
Liver	2[e]	0.0024	0.0013	0.0218	0.0867	604
	6	0.0093	0.0031	0.0062	0.1462	634
	15	0.0061	0.0029	0.005	0.1586	610
Lung	2[f]	0.0292	0.0033	0.0205	0.0631	530
	6	0.0313	0.0101	0.0022	0.1449	1371
	15	0.0149	0.0051	0.0013	0.2538	2232
Spleen	2	0.0235	0.0023	0.0104	0.3565	1042
	6	0.014	0.002	0.0083	0.3902	970
	15	0.0094	0.0023	0.0068	0.3644	779
Testes	2	0.0027	0.0004	0.001	2.4501	6905
	6	0.0024	0.0023	0.0036	1.74	600
	15	0.0021	0.0019	0.007	0.3236	510

[a]Data from 240–480 min ignored.
[b]Data from 1440 min ignored.
[c]Data from 180–480 min ignored.
[d]Data from 120–480 min ignored.
[e]Data from 120 min ignored.
[f]Data from 120 and 480 min ignored.
[g]Derivation of the kinetic parameters is detailed in the Methods Section.

Because of the method of dosing, there was the possibility that some of the dose would not be delivered into the vein. The residue values for each tissue for each animal were compared to the means of all four animals, and if it was determined that residue values were uniformly low, then all values from that animal were removed. For example, 1 rat in the

10-min group at the 6-mg/kg dose level displayed residues far below those found in the other animals within the same group. In this case, it was considered to be highly probable that the dose was delivered extravenously. In the 6-mg/kg dose level, 5 animals were also removed for the same reason. These were 2 animals from each of 45- and 60-min groups and one from the 240-min group.

In order to facilitate the fitting of the data, any temporary increase in an otherwise steadily declining description of concentration over time was not included in the fitting of the model. This means that any potential enterohepatic recycling would not be fitted by these models. In the case of blood from animals dosed at 6 mg/kg, values between 240 and 480 min were ignored. To fit the kidney data, all dose levels had values removed between 180 and 480 minutes for the 2- and 15-mg/kg groups and between 120 and 480 minutes for the 6-mg/kg dose level. Certain other values were ignored for the purposes of parameter generation if they were identified as outliers using the range/standard deviation test as described

TABLE 12. Rate coefficients, apparent volume of distribution (A.V.D.), and half-lives of BaP in tissues of rats after iv administration of the test substance

Tissue	Dose (mg/kg)	k_{12}[d] (min^{-1})	k_{21} (min^{-1})	k_{el} (min^{-1})	A.V.D. (L)	$t_{1/2}$ (min)
Blood	2	0.0428	0.0483	0.0475	0.487	36
	6	0	0.0282	0.0282	0.9077	25
	15	0.0031	0.1304	0.0164	0.4859	408
Brain	2	0.0024	0.0004	0.0058	0.0778	2326
Adipose	2	0.0009	0.0005	0.0002	0.4283	239
	6	1.0E-6	0.0007	0.0008	1.2005	945
	15	0	0.0009	0.0008	0.5065	781
Heart	2[a]	0.0099	0.0687	0.0373	0.1494	25
Kidney	2	0.0039	0.0016	0.0232	0.1792	498
	6	0.0032	0.0017	0.0244	0.3014	456
	15	0.0012	0.0016	0.0136	0.4224	389
Liver	2[b]	0.0411	0.0504	0.0639	0.1114	28
	6	0.0015	0.0023	0.033	0.1603	163
	15	0.0009	0.0026	0.0203	0.4472	281
Lung	2[c]	0.0015	0.0005	0.0138	0.3949	418.5
	6	0.0468	0.0133	0.0014	0.1421	2215.6
	15	0.0003	0.0002	0.0003	0.3677	10164
Spleen	2	0.0002	0.0184	0.0313	3.598	38

[a]Data from 120 min ignored.
[b]Data from 120 min ignored.
[c]Data from 120, 300, and 480 min ignored.
[d]Derivation of the kinetic parameters is described in the Methods section.

TABLE 13. Rate coefficients, apparent volume of distribution (A.V.D.), and half-lives estimated of BaP metabolites in rat tissues

Tissue	Dose (mg/kg)	k_{12}[c] (min^{-1})	k_{21} (min^{-1})	k_{el} (min^{-1})	A.V.D. (L)	$t_{1/2}$ (min)
Blood	2	0.0592	0.0289	0.0033	0.868	666
	6	0.0343	0.0192	0.0023	0.643	848
	15	0.0007	0.0028	0.0013	1.598	179
Adipose	6	0.0004	0.0007	0.0008	1.201	945
	15	0.0001	0.0008	0.0003	0.974	796
Heart	2[a]	0.0026	0.0034	0.0026	2.105	589
Kidney	2	0.0001	0.0001	0.0009	0.369	5132
	6	0.0001	0.0027	0.0011	0.322	241
	15	0	0.001	0.001	0.308	692
Liver	2[a]	0.0022	0.0012	0.0169	0.135	651
	6	0.0027	0.0024	0.0027	0.393	728
	15	0.0053	0.0035	0.0038	0.223	598
Lung	2[b]	0.0609	0.0025	0.0429	0.037	688
	6	0.0033	0.0006	0.0004	0.772	11511
	15	0.0129	0.0039	0.0218	0.433	295
Spleen	2	0.0248	0.0025	0.0099	3.388	1013

[a]Data from 120 min ignored.
[b]Data from 120, 300, and 480 min ignored.
[c]Derivation of the kinetic parameters is detailed in the Methods section.

by Grubbs (1969). Because of the variable nature of some of the data and difficulties in fitting some models with certain data points, values removed as possible outliers were confirmed as such by calculating the residual from the final fitted model and applying the test for outliers.

It was expected that for a given tissue, the values of A and B would increase monotonically with dose; this was the case for all tissues for ^{14}C activity, but was not the case for "free" BaP values for blood, fat, and liver, but was true for kidney. In the application of model 1, at zero time the model reduces to $A + B$, which is an estimate of the concentration when the dose is given, assuming instantaneous dispersal to the blood or tissue. Again, it would be expected that initial concentrations would rise with increasing dose for richly perfused tissues like liver, lung and spleen. The lung tissue did not follow this pattern, with the 2-mg/kg dose level having a higher concentration of ^{14}C-equivalents than the 6-mg/kg dose level at the first sampling time. Variations like these were also seen with the metabolite data, as they were merely residuals obtained from the total and "free" BaP data. In some tissues, the data were quite variable and the parameter estimates had large asymptotic standard errors, which could be due, in part, to interanimal variability.

DISCUSSION

It has been reported that administration of similar doses of pyrene to rats has led to approximately equal excretion of ^{14}C-label via urine and feces after 6 d (Withey et al., 1991). In the present study for BaP, fecal excretion was the dominant route, with the cumulative ^{14}C-activity level being between 4 and 10 times higher than that found in urine 32 h after dosing. These findings are in agreement with the early study by Kotin et al. (1959) and more recent studies on the fate of inhaled BaP by Sun et al. (1982) and percutaneous absorption of BaP by Sanders et al. (1984). Each of these studies found the feces to be the dominant route of excretion for BaP, probably because of biliary excretion.

Enterohepatic recycling has been shown to occur for a variety of compounds (Klaassen & Rozman, 1991) when excretion via the bile led to reabsorption from the gut and caused an increase in the blood concentration. The concentration of radioactivity in blood did show an increase at

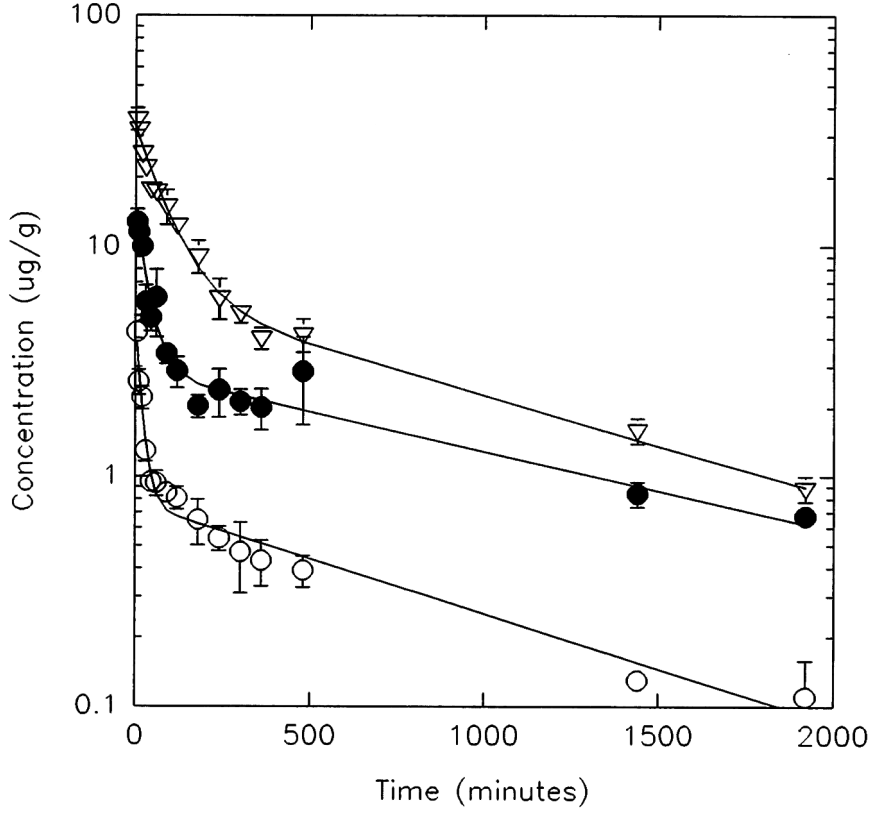

FIGURE 1. Plot of concentration of total radioactivity, as micrograms BaP equivalents per gram of blood, against time. Points are measured values, and curves are drawn using the determined kinetic parameters (○ 2 mg/kg; ● 6 mg/kg; ▽ 15 mg/kg).

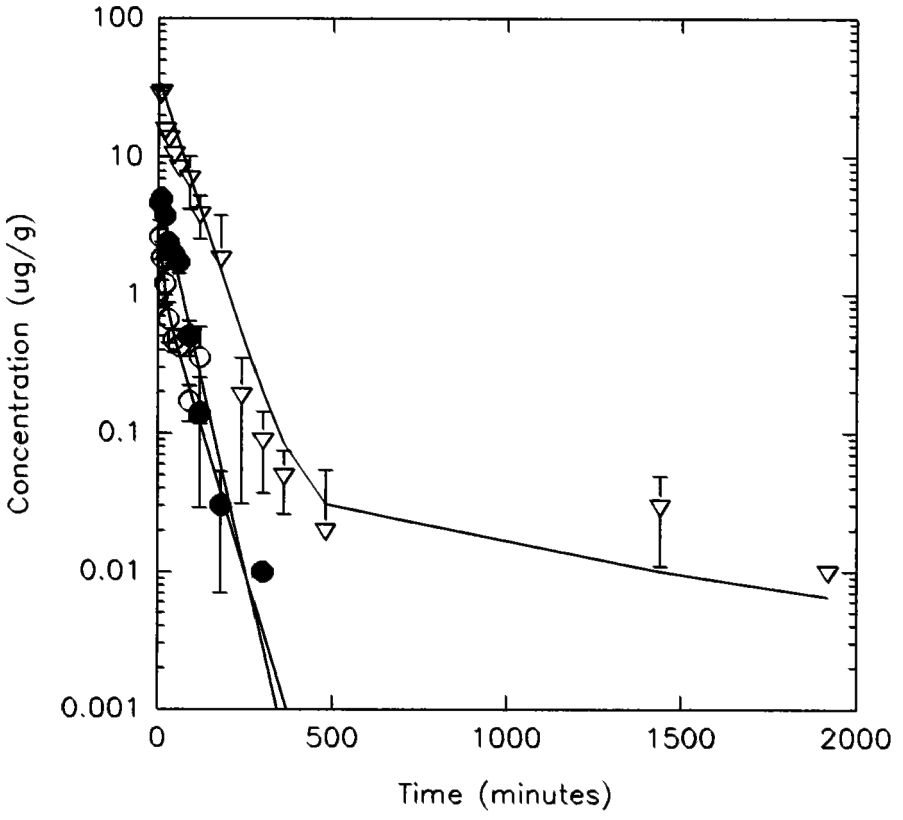

FIGURE 2. Plot of concentration of BaP as micrograms per gram of blood against time. Points are measured values, and curves are drawn using the determined kinetic parameters (○ 2 mg/kg; ● 6 mg/kg; ▽ 15 mg/kg).

480 min for the 6- and 15-mg/kg dose levels, but this was not reflected in the data for BaP and was therefore probably associated with metabolites. As shown in Figure 2, the levels of BaP for the 2 lowest dose levels became too low to measure beyond 3 h. The 15-mg/kg dose level did not show an increase in blood BaP concentration at 480 min. In Figure 1 showing the curves of total ^{14}C concentrations, the slopes of the β phase of the three dose levels were very similar, but the α phases displayed shallower slopes at higher dose levels (Table 8), which suggested nonlinearity of the kinetics with dose. This was not surprising given the magnitude of the doses and has also been described for pyrene (Withey et al., 1991). The dose levels in the present study were chosen to match those in the Withey et al. (1991) study. The dose levels are high enough to help identify nonlinearities in kinetics and saturable processes. The elimination-phase half-life for BaP in blood at a dose of 15 mg/kg was found to be about 400 min. It must be pointed out that the concentration at 1920

min was the value from only 1 animal and that at 1440 min was from 2 animals. These data points were critical in determining the slope of the elimination phase and ideally should have been available from the means of a larger number of animals (Table 7). The adipose concentration of 5.8 μg/g tissue at 1920 min was likely high enough to provide the low levels of BaP in blood even that long after injection.

Inspection of the curves showing the concentrations of ^{14}C-equivalents in liver (Figure 3) revealed a definite nonlinearity of the kinetics. Although the three dose levels displayed almost identical terminal-phase half-lives (Table 11), the lowest dose level was characterized by a greater elimination rate coefficient than the other two level (Table 11). This was probably due to a saturable process and was clearly evident only in the ^{14}C concentrations in the liver. When the ^{14}C-equivalents values for liver shown in Tables 2, 3, and 4 are normalized for dose and considered as percent of the administered dose, all 3 dose levels are very similar until the 120-min sampling interval. At that time, the 6- and 15-mg/kg dose

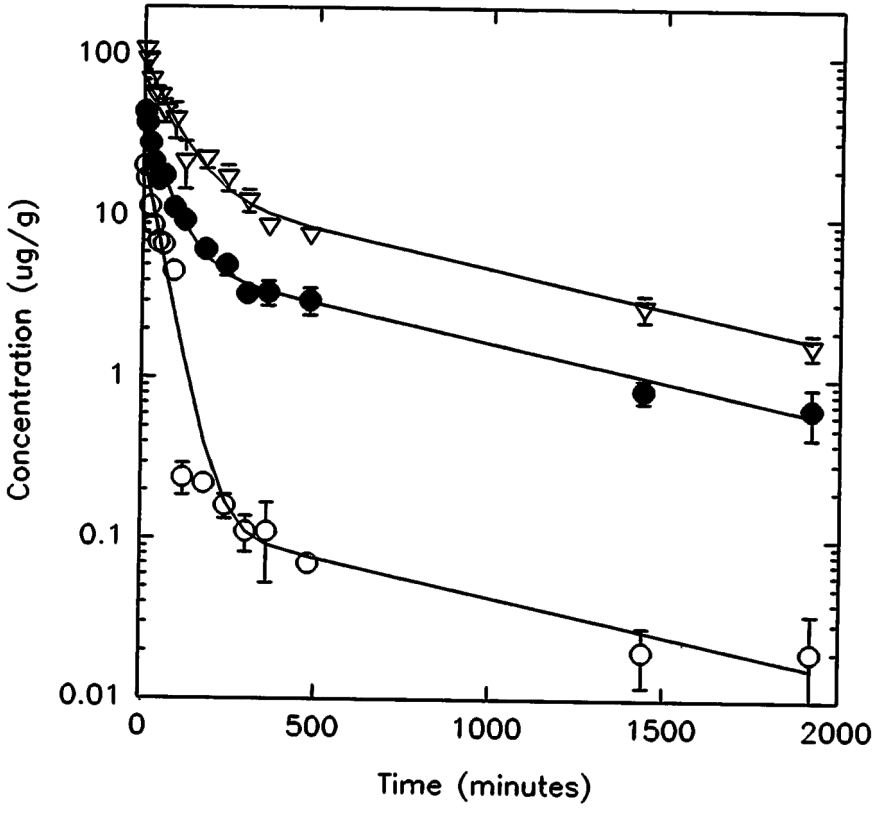

FIGURE 3. Plot of concentration of total radioactivity, as micrograms BaP equivalents per gram of liver, against time. Points are measured values, and curves are drawn using the determined kinetic parameters (○ 2 mg/kg; ● 6 mg/kg; ∇ 15 mg/kg).

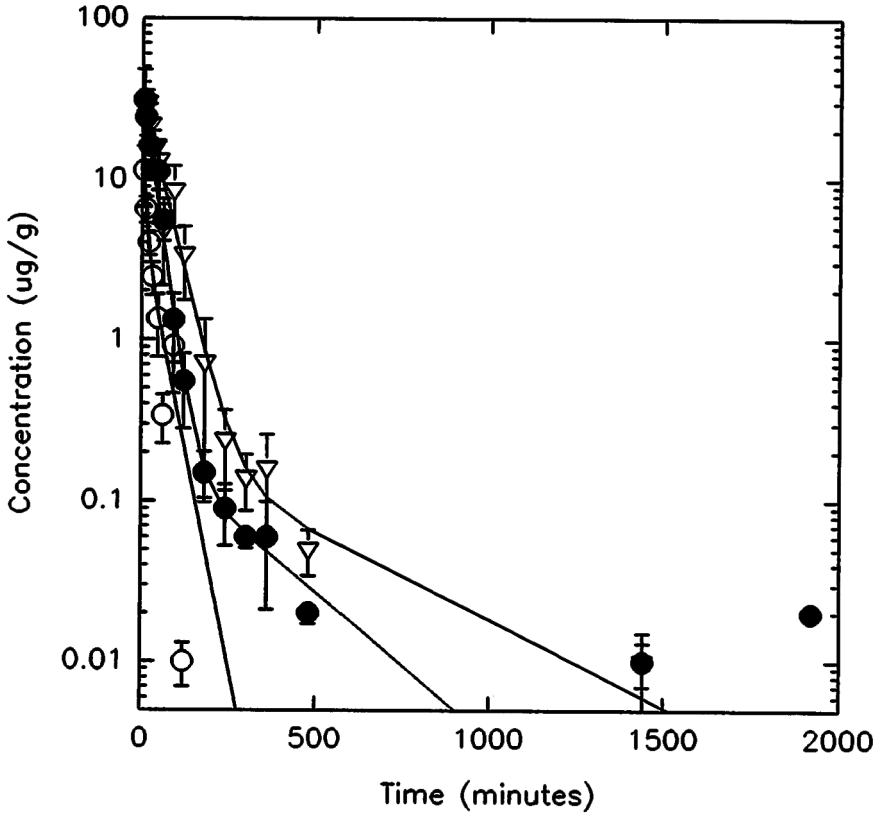

FIGURE 4. Plot of concentration of BaP as micrograms per gram of liver against time. Points are measured values, and curves are drawn using the determined kinetic parameters (○ 2 mg/kg; ● 6 mg/kg; ∇ 15 mg/kg).

groups had residue levels of 6.4% of the dose, while the 2-mg/kg dose group had residues of 0.5%. That difference was maintained through to the 32-h sampling interval. The same effect was implied in the curves for BaP in the liver, but as the last measurable value for the 2-mg/kg dose level was at 120 min, the effect cannot be confirmed. A saturable kinetic process for BaP was demonstrated previously by Kotin et al. (1959). Increasing amounts of radiolabeled BaP were administered intravenously to bile-duct-cannulated rats and the radioactivity in bile, over a period of time, was measured. A limit of 150 μg of administered BaP was reached, above which there was no appreciable increase in biliary radioactivity. Less than 1% of the radioactivity was due to the parent compound. This indicated that the metabolic processes converting BaP to bile-soluble metabolites were saturated. More recently, Weyand and Bevan (1987) investigated the effect of dose on the biliary excretion rate of radiolabel from tritiated BaP and found that the percent of an intratracheally instilled

dose found in the bile decreased with amount given, which also supported a saturable process in liver. Table 1 shows urinary and fecal radioactivity from animals killed 480, 1440, and 1920 min postdosing, and the values showed no dose dependence. This was probably a consequence of the large doses employed, which were all higher than those used in the studies just discussed. Also, there was the possibility of reabsorption, although this was not reflected in the blood data obtained in this study.

After an initial rapid decline, the kidney tissue showed a distinct increase for all three doses in the concentration of radioactivity beginning about 3 h postdosing (Tables 2, 3, and 4). This phenomenon was also described in an inhalation study conducted by Mitchell (1982) in which the radioactivity in kidney peaked about 6 h after exposure. In the present study, this increase was not reflected in the concentration of BaP itself, at any of the doses (Tables 5, 6, and 7). Therefore, the increase in concentration of radioactivity was probably indicative of the production of

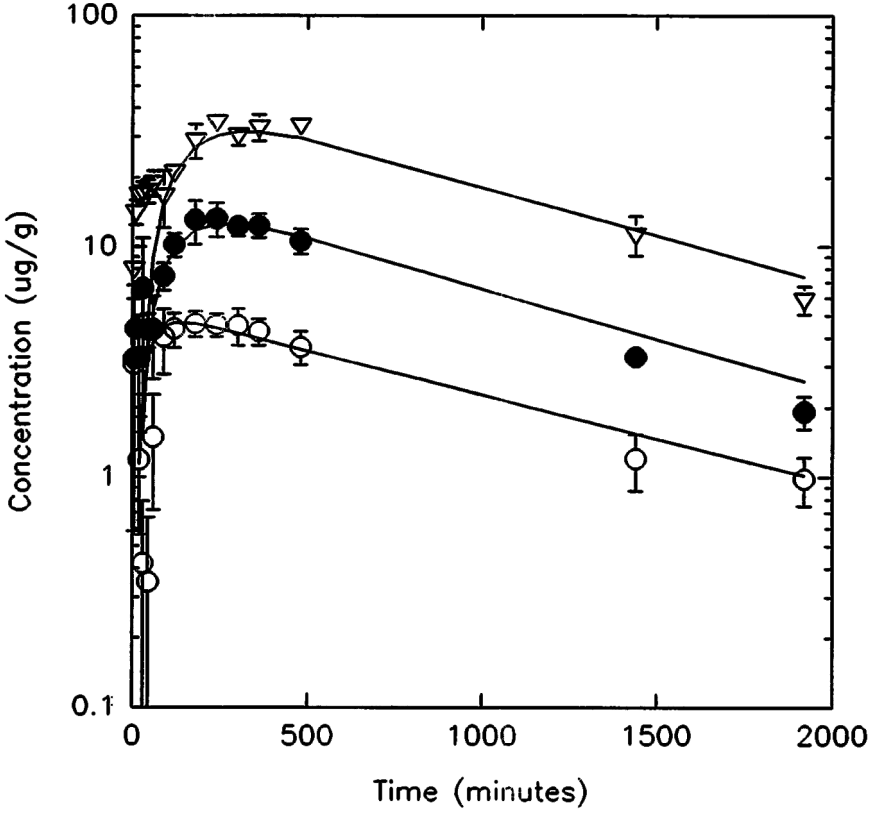

FIGURE 5. Plot of concentration of BaP metabolites in kidney (concentration of total radioactivity minus the concentration of BaP) against time. Points are measured values, and curves are drawn using the determined kinetic parameters (○ 2 mg/kg; ● 6 mg/kg; ▽ 15 mg/kg).

metabolites and their appearance in the kidney prior to excretion in the urine.

Not surprisingly, adipose tissue displayed a distinct uptake phase for both total radioactivity and for BaP itself, due to its poor vascularity, relative to other organs, and its ability to act as a sink, with high, maximal levels attained about 5–6 h postdosing. The elimination rates were dose independent in the case of free BaP and radiolabel, but the values for BaP concentration at the 2 mg/kg dose level seemed anomalously high relative to those at 6 mg/kg, when compared to the results for ^{14}C-equivalents. We have no explanation for this, and further work is required.

The lung tissue had some of the highest measured concentrations of ^{14}C-equivalents, especially at the initial sampling time. Lung has been shown to be an organ of xenobiotic metabolism and has been shown to be capable of metabolizing inhaled BaP (Mitchell, 1983), but, in general, to be less involved than liver. Roth and Vinegar (1990) investigated the role of the lung in xenobiotic metabolism and developed a physiologically based pharmacokinetic (PBPK) model for BaP. It was noted that the lung is the only organ to receive the total cardiac output and it is the organ of first exposure to xenobiotics in inhalation, intravenous, intramuscular, and subcutaneous studies. Their PBPK model suggested that the lungs of rats pretreated with 3-methylcholanthrene contributed substantially to the metabolic clearance of BaP when the dose was administered intravenously. The initial, very high values for ^{14}C-equivalents in lung in the present study (Tables 2, 3, and 4) may be due to the extensive perfusion of the lung and an effective transfer of BaP to lung from blood. Bevan and Weyand (1988) developed a compartmental model for the disposition of intratracheally instilled tritiated BaP and described the extremely rapid removal of radioactivity from lung to blood, and also predicted considerable recycling of radioactivity back to the lung as a consequence of a high rate constant for the transfer of labeled material from blood to lung. No distinction was made between BaP and metabolites. The lung data from this study could not confirm this recycling, in part because of the different method of dosing and in part because the values over time were quite scattered (Tables 2, 3, and 4). Not only was there substantial scatter from one time interval to the next, but there was also a large variability at each time interval as shown by the large standard deviations for ^{14}C-equivalents for all dose levels, which implies the possibility of large interanimal variation (Tables 2, 3, and 4).

Covalent binding of BaP-derived radioactivity to lung tissue has been suggested by Mitchell (1983) and Weyand and Bevan (1987). This latter study found 9% of the dosed radioactivity in lung per gram of tissue at 6 h postdosing. When ^{14}C data from the present study are considered in the same fashion, there is no large percentage of the dose found in lung at any of the sampling intervals. However, if the residue values as micrograms per gram are considered relative to other tissues, then lung does

appear to be a preferred organ for disposition of radioactivity and BaP. Tables 5–7 show BaP concentrations, and the lung values are higher at each dose level than any tissue except adipose at 2 mg/kg, where these values have been questioned already. Although not quite so consistent, this trend is present in the ^{14}C data as well (Tables 2–4). This is also reflected in the relatively large parameter estimates of A and B for lung for BaP (Table 9) and ^{14}C (Table 8) and in the long half-lives for that tissue (Tables 11 and 12). Bevan and Weyand (1988) reported a very rapid uptake into blood of an intratracheally instilled dose of tritiated BaP at a dosage level of about half the lowest dose in the present study. This rapid uptake indicated a favorable transfer coefficient from lung to blood. The present study has found substantial concentrations of both radioactivity and BaP, at the later sampling intervals, in lung in the presence of far lower concentrations in blood, which supports binding of these species within lung.

Heart also displayed some high ^{14}C-equivalents concentrations, especially shortly after injection. HPLC analysis of heart tissue for BaP concentration after dosing at 2 mg/kg showed that the radioactivity was almost exclusively in the form of BaP itself (data not shown). These high concentrations are due to the high volume of blood to which heart is exposed.

In summary, this study allowed an examination of the kinetics and disposition of BaP and radiolabel derived from ^{14}C-BaP in the rat after intravenous administration. Pharmacokinetic parameters have been derived that describe this disposition in blood, liver, lung, kidney, and adipose for BaP, and lung, spleen, brain, and testes for ^{14}C-equivalents of BaP.

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