Effects of Long-Term Exposure to Environmental Levels of Polychlorinated Biphenyls on Pharmacokinetics of Pentobarbital in Rats

C. K. CHU, V. J. STELLA ˣ, J. V. BRUCKNER *, and W. D. JIANG *

Abstract □ The pharmacokinetics of pentobarbital, 30 mg/kg iv, were studied in untreated rats and rats pretreated with 1, 5, and 25 ppm of polychlorinated biphenyls in food for up to 140 days. Environmental contaminants may contribute to variations in metabolic rates of drugs by causing enzyme induction. The objective of this work was to quantitate the effects of environmental levels of the contaminant and enzyme inducer, a polychlorinated biphenyl, on the pharmacokinetics of pentobarbital, a drug whose primary elimination route is liver metabolism. The pharmacokinetics of pentobarbital in rats could be fit to a biexponential equation of the type $C_p = Ae^{-\alpha t} + Be^{-\beta t}$. After 35 days of pretreatment, only the 25-ppm-treated rats showed any significant acceleration of pentobarbital elimination. At the 70- and 140-day samplings, both the 5- and 25-ppm pretreatments showed significant acceleration of pentobarbital elimination. There were no significant effects on A, α, B, and V_d for any pretreatment. The β-values for the 25-ppm-pretreated rats reached a constant value from the 35-day pretreatment period onward. A calculation of total body clearance suggested that pentobarbital elimination in those rats had approached portal blood flow rate-limited metabolism.

Keyphrases □ Biphenyls, polychlorinated—effects on pharmacokinetics of pentobarbital, rats □ Pharmacokinetics—pentobarbital, effects of polychlorinated biphenyls, rats □ Pentobarbital—pharmacokinetics, effects of polychlorinated biphenyls, rats □ Contaminants, environmental—polychlorinated biphenyls, effects on pharmacokinetics of pentobarbital, rats □ Sedatives—pentobarbital, pharmacokinetics, effects of polychlorinated biphenyls, rats

Polychlorinated biphenyls have become a major environmental contaminant because of their extreme stability and lipophilicity (1–4). Polychlorinated biphenyls have been found in human tissues (5), and an outbreak of chloracne in Japan (6) has been attributed to their accumulation in tissue. Adverse effects on reproduction in rats (7) and monkeys (8) have also been associated with polychlorinated biphenyl ingestion. Polychlorinated biphenyls induce the activity of the hepatic mixed-function oxidase system (1, 4, 9–14), and several studies showed that polychlorinated biphenyl-treated animals have enlarged livers (10, 15). However, most previous studies were carried out on animals pretreated with high levels of polychlorinated biphenyls over a relatively short time.

Rational drug therapy requires an understanding of the pharmacokinetics of drug substances and the effects that dietary, genetic, and environmental factors, as well as disease states, have on pharmacokinetics. The pharmacokinetics of pentobarbital in rats *per se* have been studied (16, 17). The objective of this study was to quantitate the effects of environmental levels of polychlorinated biphenyls on the pharmacokinetics of pentobarbital, a drug whose primary elimination route is liver metabolism (17).

This report appears to be the first to present a full pharmacokinetic study of the effects of an environmental contaminant on the distribution and elimination kinetics of a drug. The study deals with the effect of exposure to a commercial polychlorinated biphenyl for up to 140 days. The pharmacokinetics were studied in control groups and groups exposed to three different levels of polychlorinated biphenyls[1]. The three levels were 1, 5, and 25 ppm in food, representing submaximum, maximum, and above maximum Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) recommended allowable limits in most food products (18), respectively.

EXPERIMENTAL

Male, Sprague–Dawley rats[2], ~100 g, were housed in stainless steel cages in groups of three or four. Tap water was available *ad libitum*. The rats were fed standard powdered laboratory rat chow diet[3] for 1 week before a special diet was started. The animals were divided into four groups and were fed the lab chow in powdered form containing 0, 1, 5, or 25 ppm of the polychlorinated biphenyl.

The powdered diet was prepared by dissolving appropriate quantities of polychlorinated biphenyl in acetone and mixing this solution thoroughly with a small amount of ground chow. After allowing the acetone to evaporate, this contaminated chow was mixed by geometric dilution with increasing amounts of ground chow until the desired concentrations of polychlorinated biphenyl were achieved.

Four rats from each group were studied at the end of each period (35, 70, and 140 days). Each rat received 30 mg/kg iv of pentobarbital sodium *via* the dorsal vein of the penis. Blood sampling from the tail vein was started immediately after drug administration. About 250 μl of blood was collected in a heparinized microcapillary tube at specified timed intervals (Table I). The time required for the animal to regain its righting reflex was also noted. The blood was immediately centrifuged; the plasma was removed, placed into a test tube, and kept frozen until analyzed. All plasma samples were analyzed within 1 month (19).

The plasma concentration was analyzed using GC. This method was a modification of the procedure of Ehrnebo *et al.* (20). To each 100-μl plasma sample, 25 μl of internal standard (80 μg/ml of secobarbital sodium in methanol) and 0.1 ml of 0.05 *N* sodium hydroxide were added. The mixture was extracted with 1.0 ml of water-saturated ether on a mixer[4] for about 1 min. After centrifugation, the organic phase was discarded. The aqueous phase was then acidified with 1.0 ml of 4 *M* monobasic sodium phosphate and reextracted with 1.0 ml of water-saturated ether. The ether extract was then evaporated to dryness under nitrogen.

Just before injection into the chromatograph, 25 μl of 0.2 *M* trimethylanilinium hydroxide[5] was added and the sample was agitated in a mixer. An aliquot of 1.5–3.5 μl of this mixture was then injected into a gas chromatograph[6] equipped with a flame-ionization detector. The column of choice was a 1.83-m (6-ft) × 0.31-cm (0.125-in.) U-shaped glass column[7] packed with 3% OV-1 on 100–120-mesh Gas Chrom Q[8]. The following conditions were used: injector temperature, 250°; column temperature, 130°; detector temperature, 240°; nitrogen flow, 50 ml/min; hydrogen flow, 40 ml/min; and compressed air (19–23% O_2, 77–81% N_2) flow, 365 ml/min. The sensitivity was 10^{-12} amp/mv. The described procedure was used for plasma samples containing known amounts of

[1] Aroclor 1254 containing 54% (w/w) of chlorine, lot KA601, Monsanto Chemical Co., St. Louis, Mo.
[2] ARS/Sprague–Dawley, Madison, Wis.
[3] Wayne Lab-Blox, Allied Mills Inc., Chicago, Ill.
[4] Vortex Genie, Scientific Products, McGraw Park, Ill.
[5] Supelco, Inc., Supelco Park, Bellefonte, Pa.
[6] Varian Aerograph model 2100, Walnut Creek, Calif.
[7] Supelcoport, Supelco, Inc., Supelco Park, Bellefonte, Pa.
[8] Applied Sciences Labs, State College, Pa.

Table I—Plasma Sampling Times in the Study of Pentobarbital Pharmacokinetics in Rats Pretreated over Various Time Intervals with Polychlorinated Biphenyls

Days of Treatment	Sampling Time, min									
35	0	5	10	15	30	60	90	120	—	—
70	5	10	15	20	30	45	60	75	90	—
140	2	3	5	10	15	20	30	45	60	75[a]

[a] The 75-min sample was omitted for 5- and 25-ppm groups.

pentobarbital and secobarbital to produce a standard curve of the area under the peak *versus* the pentobarbital concentration (5–50 μg/ml of plasma).

The pharmacokinetics of pentobarbital in rats were fit to a two-compartment model (21) previously (16, 17). This model predicts that the data for plasma pentobarbital levels *versus* time in rats after rapid intravenous injection should show a biexponential decay. The decay curve should be described by:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 1)}$$

where C_p is the plasma level at any time t, and A, α, B, and β are parameters which were described adequately by others (21). If only a single exponential decay is noted because of the sampling schedule or because of a rapid distribution phase, then the plasma concentration *versus* time curve will be given by:

$$C_p = Be^{-\beta t} \quad \text{(Eq. 2)}$$

In the present case, for the 35- and 70-day pretreatments, the data were fit to a one-compartment model (Eq. 2) because plasma sampling times were started too late to fit the data to a two-compartment model. Therefore, the postdistribution data for each 35- and 70-day-pretreated animal were fit to Eq. 2 utilizing the simplex method of fitting[9] (22). This approach helped generate appropriate values of β and B from which the volume of distribution extrapolated (23), $V_{d(ext)}$, and total body clearance (24), Cl_{TB}, could be calculated from Eqs. 3 and 4, respectively:

$$V_{d(ext)} = \frac{\text{dose}}{B} \quad \text{(Eq. 3)}$$

$$Cl_{TB} = \beta V_{d(ext)} \quad \text{(Eq. 4)}$$

The mean values for all calculated parameters for each pretreatment were determined and statistically compared to the same parameters for the control group using the standard Student t test. The mean values of β and B were then used to generate lines to describe the plasma pentobarbital level *versus* time curves for each group.

For the 140-day-pretreated rats, plasma samples at 2 and 3 min as well as other plasma samples (Table I) did allow the fit of the data to a two-compartment model using the simplex method of fitting (22). The mean values for A, α, B, and β as well as values for $V_{d(ext)}$, $V_{d(area)}$, and Cl_{TB} were calculated for each animal in each pretreatment. The value of $V_{d(area)}$ was calculated using:

$$V_{d(area)} = \frac{\text{dose}}{\beta\left(\frac{A}{\alpha} + \frac{B}{\beta}\right)} \quad \text{(Eq. 5)}$$

These values were compared, using the Student t test, to the same parameter for the control animals. The mean values of A, α, B, and β were then used to generate lines representing the mean plasma levels *versus* time decay curves for pentobarbital in rats pretreated over 140 days with 0, 1, 5, and 25 ppm of polychlorinated biphenyl. The plasma pentobarbital concentration at the righting reflex time for each animal was determined by substituting the righting reflex time for that animal into the equation describing the plasma level *versus* time curve for that animal.

RESULTS AND DISCUSSION

Based on the study of Lin *et al.* (16), plasma samples for pentobarbital determination for both the 35- and 70-day-pretreated rats were begun at 5 min postdosing with 30 mg of pentobarbital sodium/kg (27.22 mg of pentobarbital acid/kg). Unfortunately, this time proved to be too late

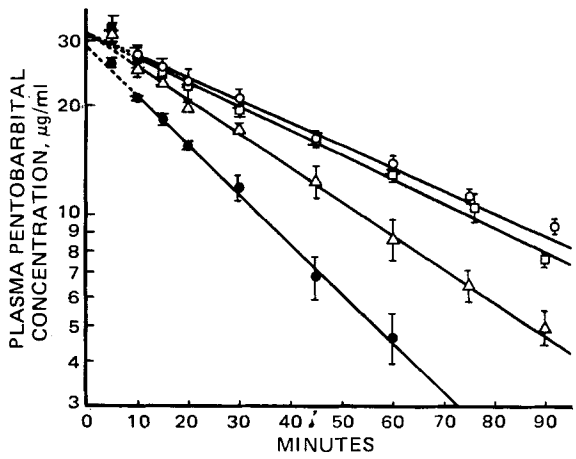

Figure 1—*Time courses of plasma concentration ± SE of pentobarbital in rats pretreated over 70 days at 1 (□), 5 (△), and 25 (●) ppm of polychlorinated biphenyls in food and in a control group of rats (○) after administration of pentobarbital sodium (30 mg/kg iv). Each point is the mean of four animals. Lines are generated utilizing pharmacokinetic parameters in Table III.*

to observe the distribution phase of the pentobarbital decay curve. Figure 1 shows the plasma level *versus* time plots for pentobarbital decay in the 70-day-pretreated rats. The solid lines are drawn by a fit to Eq. 2 utilizing the mean values of B and β for each group.

Tables II and III summarize the pharmacokinetic parameters for pentobarbital determined for the 35- and 70-day polychlorinated biphenyl-pretreated rats compared to the same parameters for the control groups. The only parameters affected by the polychlorinated biphenyl pretreatments to any significant extent were those directly related to β, the total body elimination rate constant.

At the 35-day pretreatment, β for the 1-ppm-pretreated rats was significantly smaller than β for the control and 5-ppm-pretreated rats. The cause of this apparent inhibition is unknown. There was a significant catalysis of pentobarbital elimination at 35 days in the 25-ppm-pretreated animals.

With the 70-day-pretreated animals, both the 5- and 25-ppm-pretreated animals showed a significant catalysis in pentobarbital total body elimination compared to the elimination in the control and 1-ppm-pretreated groups. These results are consistent with the shorter sleeping time in the 5- and 25-ppm-pretreated animals *versus* the controls and 1-ppm-pretreated animals.

Figure 2 shows the plasma level *versus* time plots for pentobarbital decay in the 140-day-pretreated animals. The data had been fit to a two-compartment model. Table IV summarizes the pharmacokinetic parameters for these animals. The only parameters affected by the polychlorinated biphenyl pretreatments to any statistically significant extent were those directly related to β, the total body elimination rate constant. Both the 5- and 25-ppm-pretreated animals showed a significant catalysis of pentobarbital elimination, total body clearance, and sleeping time.

A number of other observations can be made. The righting times were directly related to the plasma concentration of pentobarbital (~15 μg/ml). The β and Cl_{TB} for the 25-ppm-pretreated animals remained constant from the 35-day sampling period onward, and a similar observation can be made for the 5-ppm-pretreated animals when the results for the 70- and 140-day pretreatments are compared.

The clearance of any compound from the body whose primary mode of elimination is liver metabolism can be given by:

$$Cl_{TB} = QE \quad \text{(Eq. 6)}$$

where Q is the portal blood flow rate, and E is the extraction ratio for the liver. Literature values of Q for rats are 60 (25) and 42 (26) ml/min/kg and 1.2 ml/min/g (27) of liver. Since liver to body weights for the rats were determined[10] in the present study, the clearance value of 1.2 ml/min/g

[9] The program was developed by Dr. W. White and various graduate students of the Department of Pharmaceutical Chemistry, University of Kansas. The program was run on a Hewlett-Packard model 2100 computer. For copies of the program and further information, contact Dr. W. White.

[10] Independent *in vitro* metabolism studies were carried out on paired animals receiving the same diet and pretreated over the same time interval as the animals used in the present study. Liver weights as percent of total body weight were determined. An average liver weight of 3.75% of total body weight was used. This value of 3.75% is the percent liver weight of 25-ppm-pretreated animals. The percent liver weight for the 0-, 1-, and 5-ppm-treated animals was 3.41%.

Table II—Pharmacokinetic Parameters[a] of the Disposition of Pentobarbital in Rats Pretreated at Three Different Levels of Polychlorinated Biphenyls over 35 Days

Parameter	Pretreatment Level			
	Control	1 ppm	5 ppm	25 ppm
$\beta \pm SEM^b$, min^{-1}	0.017 ± 0.001	0.013 ± 0.001 ($p < 0.0125$)[c]	0.016 ± 0.002 (NS)	0.033 ± 0.001 ($p < 0.0025$)[c]
$t_{1/2} \pm SEM^b$, min	40.5 ± 2.1	52.4 ± 3.4 ($p < 0.0125$)[c]	39.9 ± 4.6 (NS)	20.8 ± 0.4 ($p < 0.0025$)[c]
$V_{d(ext)} \pm SEM^b$, liters/kg	0.84 ± 0.06	0.88 ± 0.05 (NS)	0.88 ± 0.03 (NS)	0.97 ± 0.02 (NS)
$Cl_{TB} \pm SEM^b$, ml/min/kg	14.3 ± 0.8	11.7 ± 0.7 ($p < 0.05$)[c]	15.7 ± 1.3 (NS)	32.5 ± 1.2 ($p < 0.0025$)[c]
Righting reflex $\pm SEM^b$, min	49.5 ± 3.9	50.7 ± 5.6 (NS)	51.5 ± 2.7 (NS)	20.2 ± 2.3 ($p < 0.0025$)[c]
C_p at righting reflex time $\pm SEM^b$, μg/ml	13.9 ± 0.9	16.0 ± 1.6 (NS)	12.5 ± 1.3 (NS)	15.2 ± 1.0 (NS)
Weight of rat $\pm SEM^b$, g	296 ± 6	272 ± 11	311 ± 10	287 ± 7

[a] Mean of four rats, except that there were only three rats in the 1- and 25-ppm groups. [b] Mean value ± *SE* of the mean. [c] Results are significantly different from the control group of animals at the level indicated.

Table III—Pharmacokinetic Parameters of the Disposition of Pentobarbital in Rats Pretreated at Three Different Levels of Polychlorinated Biphenyls over 70 Days

Parameter	Pretreatment Level			
	Control	1 ppm	5 ppm	25 ppm
$\beta \pm SEM^a$, min^{-1}	0.014 ± 0.001	0.015 ± 0.001 (NS)	0.021 ± 0.002 ($p < 0.0025$)[b]	0.031 ± 0.002 ($p < 0.0005$)
$t_{1/2} \pm SEM^a$, min	50.5 ± 1.0	48.4 ± 3.2 (NS)	33.7 ± 3.0 ($p < 0.0025$)[b]	22.8 ± 1.9 ($p < 0.0005$)[b]
$V_{d(ext)} \pm SEM^a$, liters/kg	0.88 ± 0.05	0.88 ± 0.04 (NS)	0.88 ± 0.05 (NS)	0.94 ± 0.02 (NS)
$Cl_{TB} \pm SEM^a$, ml/min/kg	12.1 ± 0.6	12.7 ± 0.8 (NS)	18.2 ± 1.0 ($p < 0.0025$)[b]	29.2 ± 1.8 ($p < 0.0005$)[b]
Righting reflex $\pm SEM^a$, min	51.7 ± 1.7	46.5 ± 1.8 ($p < 0.05$)[b]	35.7 ± 2.9 ($p < 0.0025$)[b]	22.7 ± 2.0 ($p < 0.0005$)[b]
C_p at righting reflex time $\pm SEM^a$, μg/ml	15.6 ± 1.1	15.9 ± 0.9 (NS)	15.0 ± 1.2 (NS)	14.6 ± 0.6 (NS)
Weight of rat $\pm SEM^a$, g	372 ± 17	374 ± 10	379 ± 6	367 ± 16

[a] Mean value ± *SE* of the mean. [b] Results are significantly different from the control group of animals at the level indicated.

Table IV—Pharmacokinetic Parameters of the Disposition of Pentobarbital in Rats Pretreated at Three Different Levels of Polychlorinated Biphenyls over 140 Days

Parameter	Pretreatment Level			
	Control	1 ppm	5 ppm	25 ppm
$\beta \pm SEM^a$, min^{-1}	0.016 ± 0.001	0.015 ± 0.002 (NS)	0.021 ± 0.001 ($p < 0.01$)[b]	0.031 ± 0.002 ($p < 0.0005$)[b]
$t_{1/2} \pm SEM^a$, min	43.7 ± 2.9	47.4 ± 5.9 (NS)	33.2 ± 1.3 ($p < 0.01$)[b]	22.3 ± 1.5 ($p < 0.0005$)[b]
$V_{d(ext)} \pm SEM^a$, liters/kg	0.89 ± 0.04	0.85 ± 0.05 (NS)	0.85 ± 0.03 (NS)	0.99 ± 0.10 (NS)
$V_{d(area)} \pm SEM^a$, liters/kg	0.73 ± 0.15	0.82 ± 0.05 (NS)	0.81 ± 0.03 (NS)	0.92 ± 0.07 (NS)
$Cl_{TB} \pm SEM^a$, ml/min/kg	13.1 ± 1.0	12.7 ± 0.8 (NS)	17.7 ± 1.0 ($p < 0.01$)[b]	30.7 ± 2.1 ($p < 0.0005$)[b]
Righting reflex $\pm SEM^a$, min	48.3 ± 4.8	49.5 ± 3.6 (NS)	38.0 ± 2.5 (NS)	25.0 ± 1.9 ($p < 0.01$)[b]
C_p at righting reflex time $\pm SEM^a$, μg/ml	15.2 ± 1.6	15.4 ± 0.8 (NS)	14.7 ± 1.2 (NS)	13.0 ± 0.9 (NS)
$A \pm SEM^a$, μg/ml	40.6 ± 18.7	35.2 ± 11.5 (NS)	43.2 ± 14.3 (NS)	38.7 ± 25.9 (NS)
$\alpha \pm SEM^a$, min^{-1}	0.69 ± 0.19	0.47 ± 0.14 (NS)	0.69 ± 0.19 (NS)	0.54 ± 0.16 (NS)
$B \pm SEM^a$, μg/ml	31.5 ± 1.5	32.5 ± 2.0 (NS)	32.5 ± 1.5 (NS)	28.4 ± 2.6 (NS)
Weight of rat $\pm SEM^a$, g	434 ± 7	447 ± 6	414 ± 8	422 ± 12

[a] Mean value ± *SE* of the mean. [b] Results are significantly different from the control group of animals at the level indicated.

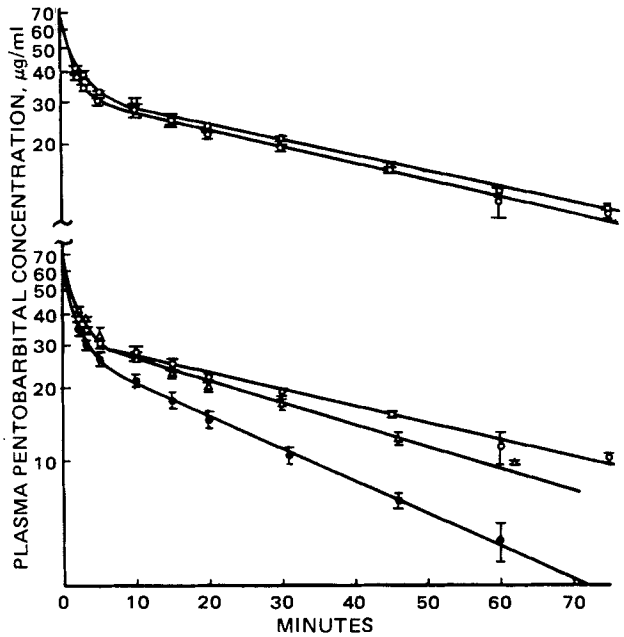

Figure 2—*Time courses of plasma concentration ± SE of pentobarbital in rats pretreated over 140 days at 1 (□), 5 (△), and 25 (●) ppm of polychlorinated biphenyls in food and in a control group of rats (○) after administration of pentobarbital sodium (30 mg/kg iv). Each point is the mean of four animals. Lines are generated utilizing pharmacokinetic parameters in Table IV.*

of liver translates to approximately 45 ml/min/kg. This value was then used for further calculations. Total body clearance in the 25-ppm-pretreated animals was calculated to be approximately 30 ml/min/kg. The extraction constant for the liver is then 0.67. This figure is only approximate in that the potential effect of enzyme induction on liver blood flow was not corrected for.

It is obvious, therefore, that two possible conditions can explain the constant pentobarbital clearance value for the 25-ppm-pretreated rats between 35 and 140 days: (*a*) the extraction constant reached a constant value and this constant value was a function of the amount of liver enzyme inducer, and/or (*b*) the clearance approached portal blood flow rate-limited metabolism. The second explanation is not totally valid in that an extraction constant of >0.8 is usually taken as portal blood flow rate-limited metabolism (28). The fact that a constant clearance was also seen in the 5-ppm-pretreated animals (70- and 140-day pretreatment) is more consistent with the first explanation that a constant E value was reached. Mechanistically, this can be explained by the hypothesis that the liver simply synthesized sufficient microsomal enzyme to prevent accumulation of the contaminant. Residues of polychlorinated biphenyls in body tissues of rats have been demonstrated to remain relatively constant on long-term feeding of similar dietary levels of polychlorinated biphenyls (29). The 1-ppm pretreatment with the polychlorinated biphenyl appeared to have no significant long-term effects on pentobarbital pharmacokinetics, suggesting that a critical mass of contaminant was needed to produce liver microsomal enzyme stimulation; *i.e.*, normal liver enzyme levels were capable of handling the 1 ppm of polychlorinated biphenyl.

Obviously, it is impossible to carry out controlled experiments of the effects of low levels of polychlorinated biphenyls on human liver microsomal enzyme activity. The FDA and EPA currently allow an upper limit of 5 ppm of polychlorinated biphenyls in most food substances (18). The present study demonstrated that 5 ppm of a polychlorinated biphenyl in food can, over a period greater than 35 days, induce liver microsomal metabolism of pentobarbital in rats as measured by the shorter half-lives of pentobarbital. At 5 and 25 ppm, the polychlorinated biphenyl stimulated pentobarbital elimination as measured by increased β and Cl_{TB} values, decreased half-lives, and shorter righting reflex times. The polychlorinated biphenyl did not significantly affect the distribution kinetics or the volume of distribution of pentobarbital, suggesting strongly that its primary effect was to induce liver metabolism *via* enzyme stimulation[10].

REFERENCES

(1) R. W. Riseborough, P. Rieche, D. B. Peakall, S. G. Herman, and M. N. Kirven, *Nature (London)*, **220**, 1098 (1968).

(2) S. Jensen, *New Sci.*, **32**, 612 (1966).

(3) F. J. Biros, A. C. Walker, and A. Medberg, *Bull. Environ. Contam. Toxicol.*, **5**, 317 (1970).

(4) J. L. Lincer and D. B. Peakall, *Nature*, **228**, 783 (1970).

(5) A. R. Yobbs, *Environ. Health Perspect.*, **1**, 79 (1972).

(6) M. Kuratsune, T. Yoshimura, J. Matuszaka, and A. Yamguchi, *ibid.*, **1**, 119 (1972).

(7) R. E. Linder, T. B. Gaines, and R. D. Kimbrough, *Food Cosmet. Toxicol.*, **12**, 63 (1974).

(8) J. R. Allen, L. A. Carstens, and D. A. Barsotti, *Toxicol. Appl. Pharmacol.*, **30**, 440 (1974).

(9) C. L. Litterst, T. M. Farber, A. M. Baker, and E. J. VanLoon, *ibid.*, **23**, 112 (1972).

(10) D. R. Bickers, L. C. Harber, A. Kappas, and A. P. Alvares, *Res. Commun. Chem. Pathol. Pharmacol.*, **3**, 505 (1972).

(11) D. C. Villeneuve, D. L. Grant, W. E. J. Philips, M. L. Clark, and D. J. Clegg, *Bull. Environ. Contam. Toxicol.*, **6**, 120 (1971).

(12) J. V. Bruckner, K. L. Khanna, and H. H. Cornish, *Toxicol. Appl. Pharmacol.*, **24**, 434 (1973).

(13) *Ibid.*, **28**, 189 (1974).

(14) J. V. Bruckner, K. L. Khanna, and H. H. Cornish, *Food Cosmet. Toxicol.*, **12**, 323 (1974).

(15) D. C. Grant, W. E. J. Philips, and D. C. Villeneuve, *Bull. Environ. Contam. Toxicol.*, **6**, 102 (1971).

(16) Y. J. Lin, S. Awazu, M. Hanano, and H. Nogami, *Chem. Pharm. Bull.*, **21**, 2749 (1973).

(17) F. W. Ossenberg, M. Peignoux, D. Bourdiau, and J. P. Benhamou, *J. Pharmacol. Exp. Ther.*, **194**, 111 (1975).

(18) *Federal Register*, **39**, 42727 (Dec. 6, 1974).

(19) R. B. Smith, L. W. Dittert, W. O. Griffen, Jr., and J. T. Doluisio, *J. Pharmacokinet. Biopharm.*, **1**, 5 (1973).

(20) M. Ehrnebo, S. Agurell, and L. O. Boreus, *Eur. J. Clin. Pharmacol.*, **4**, 191 (1972).

(21) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, pp. 57–127.

(22) S. N. Deming and S. L. Morgan, *Anal. Chem.*, **45**, 278A (1973).

(23) S. Riegelman, J. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 128 (1968).

(24) M. Rowland, *ibid.*, **61**, 70 (1972).

(25) J. Grayson and D. Mendel, "Physiology of the Splanchnic Circulation," Williams & Wilkins, Baltimore, Md., 1965, p. 74.

(26) S. S. Steiner and C. E. Mueller, *Circulation Res.*, **14**, 99 (1961).

(27) E. L. Dobson and H. B. Jones, *Acta Med. Scand.*, **144**, 34, Suppl. 273 (1952).

(28) G. R. Wilkinson, *Ann. Rev. Pharmacol.*, **15**, 11 (1975).

(29) D. L. Grant, C. A. Moodie, and W. E. J. Philips, *Environ. Physiol. Biochem.*, **4**, 214 (1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 5, 1976, from the *Departments of Pharmaceutical Chemistry and Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045.*

Accepted for publication April 26, 1976.

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, New Orleans meeting, April 1976.

Supported by University of Kansas General Research Funds and an HSAA award to V. J. Stella and J. V. Bruckner.

* Present address: Department of Pharmacology, Medical School, University of Texas Health Science Center at Houston, Houston, TX 77030.

ˣ To whom inquiries should be directed (at the Department of Pharmaceutical Chemistry).