Effects of Short-Term Dietary Exposure to Polychlorinated Biphenyls on Pharmacokinetics of Intravenous Pentobarbital in Rats

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Abstract [] The intravenous pharmacokinetics of pentobarbital (30 mg/kg as pentobarbital sodium) in rats were studied at 0, 1, 2, 3, and 10 days after pretreatment with 0, 1, 5, 25, and 125 ppm of polychlorinated biphenyls in food. The polychlorinated biphenyls then were removed from the food, and the residual effects of the exposure on pentobarbital pharmacokinetics were studied at 15, 25, 45, and 70 days after initiation of the polychlorinated biphenyl exposure. The pharmacokinetics of pentobarbital were approximated to a one-compartment model. After pretreatment at 1 and 5 ppm for up to 10 days, all pentobarbital pharmacokinetic parameters obtained were comparable to control values. Pretreatment at 125 ppm significantly reduced the biological half-life and raised the total body elimination rate constant, total body clearance, and intrinsic clearance of pentobarbital after a 1-day exposure; all parameters apparently reached a new steady-state value by Days 5-10. Enhanced pentobarbital elimination at 25 ppm was observed after a 3-day exposure, but, again, the elimination parameters appeared to have reached a steady state after 5-10 days of pretreatment. Upon removal of the polychlorinated biphenyls, the various pharmacokinetic parameters showed a lag phase *prior to a gradual return to control values. The study shows that intrinsic clearances rather than total body clearances or half-lives are more appropriate in assessing enzymatic induction in agents undergoing facile liver metabolic clearance that borders on blood flow rate dependency.

Keyphrases [] Polychlorinated biphenyls—effect of short-term dietary exposure on pharmacokinetics of intravenous pentobarbital, rats [] Pentobarbital—effect of short-term dietary exposure to polychlorinated biphenyls on pharmacokinetics following intravenous administration, rats [] Pharmacokinetics—pentobarbital, effect of short-term dietary exposure to polychlorinated biphenyls following intravenous administration, rats

The objective of this study was to observe the effects of short-term exposure to a class of environmental contaminants and hepatic enzyme inducers, polychlorinated biphenyls, on the pharmacokinetics of pentobarbital in rats. Pentobarbital, whose pharmacokinetics in rats (1) and humans (2) have been documented, is eliminated primarily *via* side-chain hydroxylation by liver microsomal enzymes (3–8). These enzyme systems are induced by exposure to polychlorinated biphenyls (9–14). Therefore, pentobarbital pharmacokinetics can be used as a probe for measuring the inducing effects of polychlorinated biphenyls on the liver microsomal enzymes in rats.

Drug pharmacokinetics can be altered by factors such as diet, age, sex, genetics, disease states, and environmental factors. Exposure to environmental contaminants such as polychlorinated biphenyls, which accumulate in human tissues (11, 15–21) and induce human hepatic enzymes (22–26), could drastically reduce the disposition half-life of drugs. Therefore, a thorough understanding of drug pharmacokinetics as affected by polychlorinated biphenyls and other environmental contaminants is useful in predicting their possible effects on drug therapy.

In a previous study (14), the effects of long-term (0–140-day) dietary exposure to a polychlorinated biphenyl

mixture (0–25 ppm) on the pharmacokinetics of intravenously administered pentobarbital in rats were quantitated. The present study examined the effects of short-term dietary exposure to polychlorinated biphenyls (0–125 ppm for 0–10 days) on the elimination kinetics of intravenously administered pentobarbital in rats. The polychlorinated biphenyls were removed from the food at the end of 10 days, and the residual effects of the exposure were followed for 70 days. This procedure allowed a time profile to be established for the inducing effects of the polychlorinated biphenyls. Some qualitative observations concerning the time profile for the alteration of pentobarbital pharmacokinetics by polychlorinated biphenyl exposure are presented. The significance of the effects of accidental human exposure to polychlorinated biphenyls also is discussed.

EXPERIMENTAL

Animals—Male Sprague–Dawley rats[1], 201–225 g, were housed in stainless steel cages in groups of four. All animals were fed standard laboratory rat food[2] for 1 week before the prepared polychlorinated biphenyl[3]-containing diet was introduced. Tap water was available *ad libitum*. Groups of animals then were put on the prepared diet for 0, 1, 2, 3, 5, 10, 15, 25, 45, or 70 days. The rats were exposed (for a maximum of 10 days) to the polychlorinated biphenyl-containing powdered rat food prepared as described previously (13, 14). For animals on the 15-, 25-, 45-, and 70-day diet schedules, the polychlorinated biphenyl-containing powdered rat food was removed after 10 days and replaced with polychlorinated biphenyl-free powdered rat food.

The polychlorinated biphenyl diet was initiated in the afternoon, and all blood sampling studies were carried out in the mornings of the specified days. For example, rats on a 1-day diet were given the polychlorinated biphenyl-containing food in the afternoon of Day 0, and the pharmacokinetics of intravenously administered pentobarbital were studied on the following morning (*i.e.*, the morning of Day 1). Similarly, rats on a 15-day diet were exposed to polychlorinated biphenyl-containing food from the afternoon of Day 0 until the afternoon of Day 10, when powdered polychlorinated biphenyl-free rat food replaced the contaminated food.

For each time period, the rats were divided into groups of eight and were fed powdered laboratory rat food containing 0, 5, 25, or 125 ppm of polychlorinated biphenyls. An additional group of eight animals fed 1 ppm of polychlorinated biphenyl-containing food was included in the 10- and 70-day studies.

Five rats from each group were studied at the end of each time period. Pentobarbital sodium[4], 30 mg/kg (equivalent to 27.7 mg of pentobarbital/kg), was administered to each rat intravenously *via* the dorsal vein of the penis (27). The blood sampling procedure was similar to that described previously (14). Blood samples were collected in microcapillary tubes pretreated with 10% sodium ethylenediaminetetraacetate at 5, 7, 10, 15, 20, 30, 45, 60, and 90 min postdosing. Rats were sacrificed after

Table I—Biological Half-Lives [a] of Pentobarbital in Rats Pretreated at Four Levels of Polychlorinated Biphenyls Compared to the Controls over Periods of up to 10 Days

Pretreatment Time, days	Control	1-ppm Pretreatment	5-ppm Pretreatment	25-ppm Pretreatment	125-ppm Pretreatment
0	50.9 ± 2.1	—	—	—	—
1	52.6 ± 6.9	—	57.9 ± 9.7 (NS)	53.1 ± 4.4 (NS)	38.6 ± 3.1 ($p < 0.02$)[b]
2	52.7 ± 10.7	—	51.2 ± 4.6 (NS)	43.3 ± 5.4 (NS)	29.6 ± 3.0 ($p < 0.01$)[b]
3	51.6 ± 3.5	—	51.5 ± 4.5 (NS)	37.9 ± 4.6 ($p < 0.01$)[b]	28.9 ± 4.8 ($p < 0.01$)[b]
5	51.1 ± 12.0	—	51.0 ± 4.5 (NS)	34.9 ± 6.9 ($p < 0.01$)[b]	23.5 ± 1.4 ($p < 0.01$)[b]
10	54.8 ± 9.9	59.2 ± 16.2 (NS)	52.2 ± 8.0 (NS)	35.5 ± 4.3 ($p < 0.05$)[b]	23.2 ± 1.8 ($p < 0.01$)[b]

[a] All values are means ± SD in minutes ($n = 5$). [b] Results are significantly different from the same-day control group at the level indicated.

Table II—Total Body Clearances [a] of Pentobarbital in Rats Pretreated at Four Levels of Polychlorinated Biphenyls Compared to the Controls over Periods of up to 10 Days

Pretreatment Time, days	Control	1-ppm Pretreatment	5-ppm Pretreatment	25-ppm Pretreatment	125-ppm Pretreatment
0	20.5 ± 3.3	—	—	—	—
1	19.9 ± 2.6	—	17.1 ± 3.2 (NS)	17.9 ± 1.8 (NS)	26.9 ± 5.9 ($p < 0.05$)[b]
2	17.1 ± 2.5	—	18.2 ± 3.4 (NS)	22.5 ± 3.3 (NS)	34.2 ± 6.0 ($p < 0.01$)[b]
3	19.9 ± 1.4	—	17.6 ± 3.4 (NS)	22.6 ± 2.4 (NS)	33.2 ± 6.7 ($p < 0.01$)[b]
5	18.7 ± 3.9	—	17.8 ± 3.0 (NS)	28.2 ± 3.8 ($p < 0.01$)[b]	42.5 ± 5.2 ($p < 0.01$)[b]
10	19.0 ± 1.3	17.2 ± 5.5 (NS)	18.5 ± 4.8 (NS)	24.4 ± 2.9 (NS)	46.8 ± 3.4 ($p < 0.01$)[b]

[a] All values are means ± SD in milliliters per minute per kilogram of body weight ($n = 5$). [b] Results are significantly different from the same-day control group at the level indicated.

each pretreatment period. The livers were removed, patted dry, and weighed.

Pentobarbital Assay—The plasma pentobarbital concentration was assayed as described previously (14) but with the following modifications. To each 100 μl of plasma sample were added 25 μl of the internal standard (0.099 mg of secobarbital/ml in methanol) and 50 μl of 4 M monobasic sodium phosphate. The mixture was extracted with 1.0 ml of water-saturated ether on a vortex mixer for 1 min. After centrifugation, the ether extract was removed to a glass-stoppered 5-ml conical centrifuge tube.

Just before injection in the gas chromatograph, 25 μl of alkaline aqueous 0.02 M trimethylanilinium hydroxide was added to the sample, which then was agitated and centrifuged. The trimethylanilinium hydroxide solution was prepared by the method of Stella (28). The ether layer was removed by aspiration, and 1–2 μl of the aqueous lower layer was injected in a gas chromatograph. Chromatographic conditions and column packings were as described previously (14). A standard curve of the peak height ratios of pentobarbital sodium to secobarbital versus plasma concentrations of pentobarbital (2–80 μg/ml) was prepared and used to determine pentobarbital in unknown samples.

Pharmacokinetic Data Analysis—In an earlier study (14), a fast distribution phase was observed in the disposition kinetics of pentobarbital in the rat. The distribution kinetics, apart from being rapid, were not affected significantly by the polychlorinated biphenyl pretreatments (14). Based on these observations, the data in this study were treated as a one-compartment model by linear least-squares fitting of the postdistribution data points on a semilog plot. The B and β values obtained from the intercept and slope of the log concentration–time curve were used to calculate the volume of distribution (extrapolated), $V_d(\text{ext})$, and the total body clearance, Cl_{TB} (14). The total body clearance of any compound that is eliminated solely via liver metabolism is given by:

$$Cl_{TB} = QE \qquad \text{(Eq. 1)}$$

where Q is the liver blood flow rate and E is the liver extraction constant (29). The extraction constant is not an independent variable but varies with Q, the fraction of unbound drug in the blood (f), and the intrinsic clearance of the drug (Cl_{int}) (29, 30). If the measured intrinsic clearance ($Cl_{int}f$) is represented by Cl_{INT}, then for a drug metabolized exclusively by the liver, the total body clearance, which approximates the hepatic clearance, can be expressed by:

$$Cl_{TB} = Q \frac{Cl_{INT}}{Q + Cl_{INT}} \qquad \text{(Eq. 2)}$$

The intrinsic clearance, defined as the clearance of a drug when there are no flow limitations (29), is a measure of the available metabolic activity of the liver and the properties of the enzyme system associated with hepatic removal. The Cl_{INT} values were calculated from Eq. 2 using blood flow rates calculated from the individual liver to body weight ratios for the particular animal and a blood flow rate of ~2 ml/min/g of liver (30).

The mean values and standard deviations for all parameters for each pretreatment were calculated and compared statistically to the same parameter for the same-day control group. Multiple means within a day were analyzed by a randomized analysis of variance. When the analysis indicated that a significant difference existed, the means of each group were compared by a two-tailed Dunnett multiple mean test (31). This test is for multiple mean comparisons to a control.

RESULTS AND DISCUSSION

Tables I and II summarize the half-lives ($t_{1/2}$) and total body clearances (Cl_{TB}) of intravenously administered pentobarbital in rats pretreated with different polychlorinated biphenyl doses over various time periods compared to the controls. The elimination rate of pentobarbital was increased significantly in rats pretreated for 1 day at 125 ppm of polychlorinated biphenyl in the food. On Day 1, $t_{1/2}$ was reduced significantly to 39 ± 3 min compared to the control group, which showed a $t_{1/2}$ of 53 ± 7 min; the $t_{1/2}$ appeared to reach a constant value of 23 ± 1 min by Days 5–10. The Cl_{TB} values also increased from 21 ml/min/kg of body weight on Day 1 and seemed to approach a constant clearance of 43–47 ml/min/kg of body weight in <1 week. No significant changes in the elimination kinetics of pentobarbital were observed for rats pretreated at the 1- and 5-ppm levels relative to control rats.

Enhanced pentobarbital elimination for rats pretreated with 25 ppm of polychlorinated biphenyls in food was observed after 2–3 days of pretreatment. The elimination parameters again began to plateau after ~5–10 days of pretreatment, as was the case with the 125-ppm polychlorinated biphenyl-pretreated rats. The $t_{1/2}$ was lowered significantly to 38 ± 5 min relative to the control value of 52 ± 4 min after 3 days of pretreatment and remained rather constant at 35 ± 4 min after ~5–10 days. The Cl_{TB} value was similarly significantly increased to 28 ml/min/kg of body weight after 5 days on a polychlorinated biphenyl-contaminated diet and appeared to begin leveling off at a clearance of 24–28 ml/min/kg of body weight after ~5–10 days of the diet.

Upon removal of the polychlorinated biphenyls from the diet after 10 days (Tables III and IV), the enhanced pentobarbital elimination as measured by $t_{1/2}$ and Cl_{TB} started to decline toward the control values after apparent time lags of ~10 and ~20 days for the 25- and 125-ppm polychlorinated biphenyl-pretreated animals, respectively. The lag time for the loss of residual effects appeared to be dose dependent. Induced enzyme activity as shown by enhanced pentobarbital elimination due to exposure to 25 ppm of polychlorinated biphenyls receded within 60 days following removal of polychlorinated biphenyls from the diet. Rats pretreated at the 125-ppm level still demonstrated an elevated, but not statistically significant, enhancement of pentobarbital elimination at 70 days, i.e., 60 days after removal of the polychlorinated biphenyls from the food.

The Cl_{TB} values (Tables II and IV) for the control group decreased slightly but insignificantly over the 70 days of the study; in the 5-ppm

Table III—Biological Half-Lives [a] of Pentobarbital in Rats Pretreated at Four Levels of Polychlorinated Biphenyls Compared to the Controls over the Periods Studied

Pretreatment Time, days	Control	1-ppm Pretreatment	5-ppm Pretreatment	25-ppm Pretreatment	125-ppm Pretreatment
10	54.8 ± 9.9	59.2 ± 16.2 (NS)	52.2 ± 8.0 (NS)	35.5 ± 4.3 ($p < 0.01$)[b]	23.2 ± 1.8 ($p < 0.05$)[b]
15[c]	55.1 ± 7.0	—	50.8 ± 4.1 (NS)	36.0 ± 5.2 ($p < 0.01$)[b]	22.3 ± 1.6 ($p < 0.01$)[b]
25[c]	54.3 ± 8.1	—	50.2 ± 8.7 (NS)	41.8 ± 7.6 ($p < 0.05$)[b]	22.5 ± 1.5 ($p < 0.01$)
45[c]	52.3 ± 8.8	—	50.5 ± 6.5 (NS)	48.2 ± 8.3 (NS)	24.0 ± 3.1 ($p < 0.01$)[b]
70[c]	56.7 ± 7.0	54.7 ± 8.3 (NS)	66.2 ± 10.3 (NS)	53.8 ± 20.6 (NS)	45.9 ± 5.7 (NS)

[a] All values are means ± SD in minutes (n = 5). [b] Results are significantly different from the same-day control group at the level indicated. [c] Animals were treated with polychlorinated biphenyls at the levels indicated for 10 days, at which time the polychlorinated biphenyl-contaminated food was replaced with polychlorinated biphenyl-free food.

Table IV—Total Body Clearances [a] of Pentobarbital in Rats Pretreated at Four Levels of Polychlorinated Biphenyls Compared to the Controls over the Periods Studied

Pretreatment Time, days	Control	1-ppm Pretreatment	5-ppm Pretreatment	25-ppm Pretreatment	125-ppm Pretreatment
10	19.0 ± 1.3	17.2 ± 5.5 (NS)	18.5 ± 4.8 (NS)	24.4 ± 2.9 (NS)	46.8 ± 3.4 ($p < 0.01$)[b]
15[c]	17.1 ± 3.9	—	16.8 ± 1.2 (NS)	26.3 ± 7.4 ($p < 0.01$)[b]	43.7 ± 3.0 ($p < 0.01$)[b]
25[c]	18.8 ± 3.5	—	18.6 ± 3.6 (NS)	25.4 ± 5.0 ($p < 0.05$)[b]	38.9 ± 1.6 ($p < 0.01$)[b]
45[c]	16.1 ± 2.1	—	16.3 ± 0.8 (NS)	22.8 ± 11.0 (NS)	33.2 ± 6.2 ($p < 0.01$)[b]
70[c]	16.0 ± 1.6	17.9 ± 2.5 (NS)	18.1 ± 3.2 (NS)	18.4 ± 6.0 (NS)	23.4 ± 5.3 (NS)

[a] All values are means ± SD in milliliters per minute per kilogram of body weight (n = 5). [b] Results are significantly different from the same-day control group at the level indicated. [c] Animals were treated with polychlorinated biphenyls at the levels indicated for 10 days, at which time the polychlorinated biphenyl-contaminated food was replaced with polychlorinated biphenyl-free food.

treated animals, the Cl_{TB} remained at a level similar to the control animals prior to exposure. This finding may suggest some minimal induction by the polychlorinated biphenyls at 5 ppm. However, as suggested by the Dunnett test, no statistically significant differences were seen between the controls and the 5-ppm treated animals at any time.

Figure 1 shows the induction time profiles for β. The dotted lines represent the range (mean ± SD) for the β values of the control animals
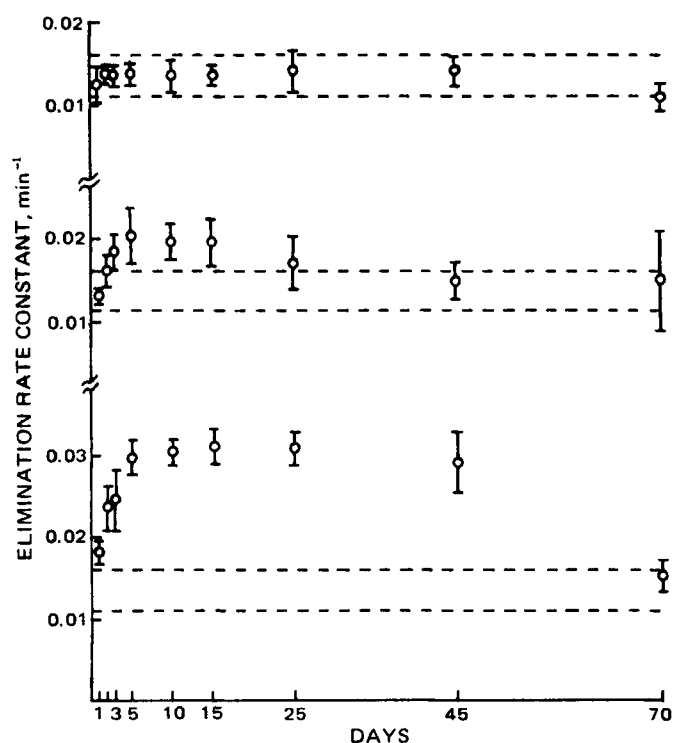

Figure 1—Induction time profiles for total body elimination rate constants (β) for animals pretreated with polychlorinated biphenyls at 5 ppm (top profile), 25 ppm (middle profile), and 125 ppm (bottom profile) over 70 days. The polychlorinated biphenyl diet was provided up to 10 days, after which time it was replaced with polychlorinated biphenyl-free food. Dotted lines represent the range of the mean control value obtained by averaging the control values at the 10 times studied (i.e., mean ± SD).

averaged over all of the time periods studied. There were no significant effects of polychlorinated biphenyl exposure as measured by β in rats pretreated at the 5-ppm level. Figure 1 also shows that the enhanced elimination of pentobarbital in rats at 25 ppm of polychlorinated biphenyls was observed only after a lag time of ~1–3 days. This pattern resembles the 5-ppm polychlorinated biphenyl-pretreated rats in the earlier study (14), which did not show any effects of polychlorinated biphenyl induction until exposure for >35 days. The increased elimination rate of pentobarbital in rats pretreated with 125 ppm of polychlorinated biphenyls was statistically significant, even after 1 day. The enhanced elimination resulted from exposure to the higher polychlorinated biphenyl doses.

After removal of the polychlorinated biphenyl-contaminated diet on Day 10, induced enzyme activity as measured by β appeared to recede completely by Day 70 in the 25-ppm polychlorinated biphenyl-pretreated rats. Despite the still elevated, but not statistically significant, elimination for pentobarbital in rats pretreated with 125 ppm of polychlorinated biphenyls at Day 70, an apparent decrease in the induced enzyme activity as measured by the same parameters was noticeable.

Body Weights and Liver Weights—No significant trend of body weight increase was observed in rats pretreated at the different levels of polychlorinated biphenyls compared to the control rats. However, the mean liver weight to body weight ratios were higher in rats pretreated at 125 ppm of polychlorinated biphenyls (Table V) throughout induction and remained elevated until ~35 days after the removal of polychlorinated biphenyls (i.e., at Day 45). The liver weights and the liver weight to body weight ratios in the 25-ppm pretreated rats were not significantly different from the control group. Dose-dependent liver weight increases were reported for mice pretreated with polybrominated biphenyls at 100- and 200-ppm dietary levels (32).

It has been shown that exposure to up to 1000 ppm of polychlorinated biphenyls for periods of up to months results in modest proliferation of the smooth endoplasmic reticulum and lipid vacuolation of liver cells but causes no cellular injury in the liver (33–36). Thus, this small increase in the liver weight and the liver weight to body weight ratio probably is an adaptive response, as suggested by Bruckner et al. (13) rather than a hepatotoxic effect. Other investigators also suggested that such changes in hepatic morphology upon exposure to polychlorinated biphenyls represent an adaptive response to eliminate foreign compounds by increasing their rate of metabolism (37). The observed enhanced rate of elimination of pentobarbital in polychlorinated biphenyl-pretreated rats probably reflects the increased activities of certain metabolic enzymes due to the exposure.

Intrinsic Clearances—Both the biological half-life and total body clearance values are limited in use as an index of intrinsic hepatic metabolism (38). The former is a complex parameter and depends on the distribution characteristics as well as the elimination of the substance under investigation. The latter can approximate hepatic clearance only

Table V—Percent of Liver Weight to Body Weight [a] for Rats Pretreated at Four Levels of Polychlorinated Biphenyls Compared to the Controls over the 10 Periods Studied

Pretreatment Time, days	Control	1-ppm Pretreatment	5-ppm Pretreatment	25-ppm Pretreatment	125-ppm Pretreatment
0	4.3 ± 0.4	—	—	—	—
1	4.0 ± 0.3	—	3.7 ± 0.3	4.0 ± 0.2	4.5 ± 0.2
2	3.4 ± 0.2	—	3.8 ± 0.2	4.2 ± 0.3	4.7 ± 0.3
3	4.3 ± 0.3	—	4.4 ± 0.4	4.2 ± 0.4	4.5 ± 0.2
5	4.6 ± 0.3	—	4.4 ± 0.2	4.6 ± 0.1	5.3 ± 0.4
10	4.3 ± 0.3	3.9 ± 0.2	3.8 ± 0.3	4.2 ± 0.3	5.6 ± 0.5
15[b]	3.8 ± 0.3	—	3.7 ± 0.3	4.1 ± 0.1	5.1 ± 0.1
25[b]	3.6 ± 0.2	—	3.9 ± 0.2	4.0 ± 0.1	4.7 ± 0.3
45[b]	3.7 ± 0.4	—	3.6 ± 0.2	3.6 ± 0.2	3.9 ± 0.6
70[b]	3.7 ± 0.1	3.4 ± 0.2	3.5 ± 0.1	3.8 ± 0.2	4.0 ± 0.5

[a] All values are means ± SD as (liver weight/body weight) × 100 (n = 5). [b] Animals were treated with polychlorinated biphenyls at the levels indicated for 10 days, at which time the polychlorinated biphenyl-contaminated food was replaced with polychlorinated biphenyl-free food.

if the drug is eliminated and metabolized exclusively by the liver. Even for pentobarbital, whose route of elimination is primarily hepatic, an estimation of the total body clearance still may not suffice as the index for intrinsic metabolic activity. As seen by examining Eq. 2, total body clearance is governed not only by the intrinsic clearance but also by the liver blood flow rate. Only when this rate is very much larger than the intrinsic clearance does the total body clearance directly reflect the intrinsic metabolic activity of the liver or the intrinsic clearance. On the other hand, when the intrinsic clearance is very large compared to the blood flow rate through the liver, the total body clearance becomes limited by the flow rate and is no longer proportional to the intrinsic clearance. To quantitate the apparent metabolic activity of the liver enzymes using pharmacokinetic parameters, estimation of the intrinsic clearance is more appropriate.

By using a literature value of 2 ml/min/g of liver for the liver blood flow rate and the measured experimental total body clearances, intrinsic clearances were estimated; the induction time profiles are presented in Fig. 2. The absence of induction of the hepatic metabolic enzymes due to 5-ppm polychlorinated biphenyl exposure as measured by the intrinsic clearances is readily seen in Fig. 2. Again, a statistically significant induction at the 25-ppm pretreatment level is seen after a 3-day lag time and an immediate (<1 day) and more pronounced induction is seen at the 125-ppm polychlorinated biphenyl pretreatment level. The absolute values of Cl_{INT} are dependent on the value used for the liver blood flow rate. Recently developed pharmacokinetic techniques (30, 39, 40) and more traditional measurements (41–44) suggested that the value of ~2

ml/min/g of liver is a reasonable estimate of the liver blood flow rate in rats. Determination of the intrinsic clearances for pentobarbital in rats using the oral and intravenous pharmacokinetic techniques (30, 39, 40) will be presented later.

If $t_{1/2}$, β, or Cl_{TB} is used as a measure of polychlorinated biphenyl induction of pentobarbital elimination, it can be seen from Fig. 1 and Tables I–IV that the percent change in the parameters relative to controls reaches a steady-state level ~5–10 days after an apparent initial lag phase. This also was seen in an earlier chronic exposure study (14). A possible explanation might be that the liver, in response to an inducer, increases the synthesis of microsomal enzymes in an amount proportional to the dose of the inducer. Since the pentobarbital total body clearance, even in the absence of induction, is a reasonably high percentage of the liver blood flow, the percentage increase in the inducing effect of the polychlorinated biphenyls is not reflected totally in the total body clearance.

For example, the steady-state total body clearance after the 10-day exposure at 125 ppm amounted to ~46 ml/min/kg. When this parameter is used as a measure of induction and compared with the control value (19 ml/min/kg), a 142% increase is indicated. If the intrinsic clearances are used instead, the results indicate an increase of 229% (80.8 ml/min/kg in treated animals versus 24.5 ml/min/kg in the controls). Thus, the total body clearances do not always reflect the large increases in intrinsic
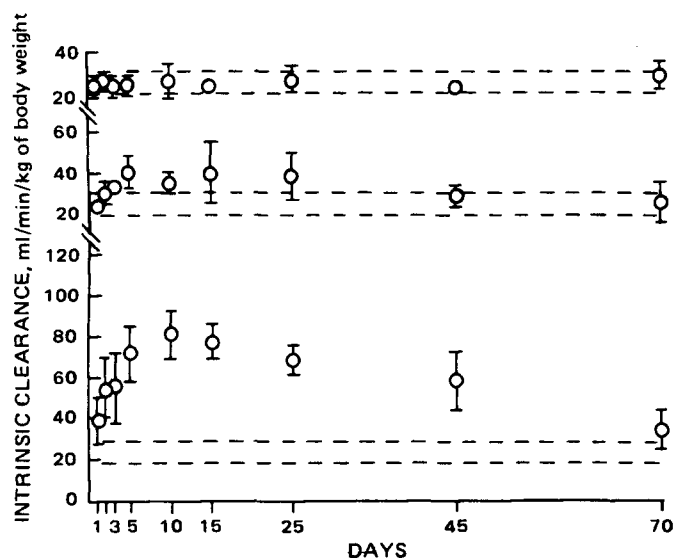

Figure 2—Induction time profiles for intrinsic clearances (Cl_{INT}) for animals pretreated with polychlorinated biphenyl at 5 ppm (top profile), 25 ppm (middle profile), and 125 ppm (bottom profile) in food over 70 days. The polychlorinated biphenyl diet was provided up to 10 days, after which time it was replaced by a polychlorinated biphenyl-free diet. Dotted lines represent the range of the mean control value obtained by averaging the control values at the 10 times studied (i.e., mean ± SD).
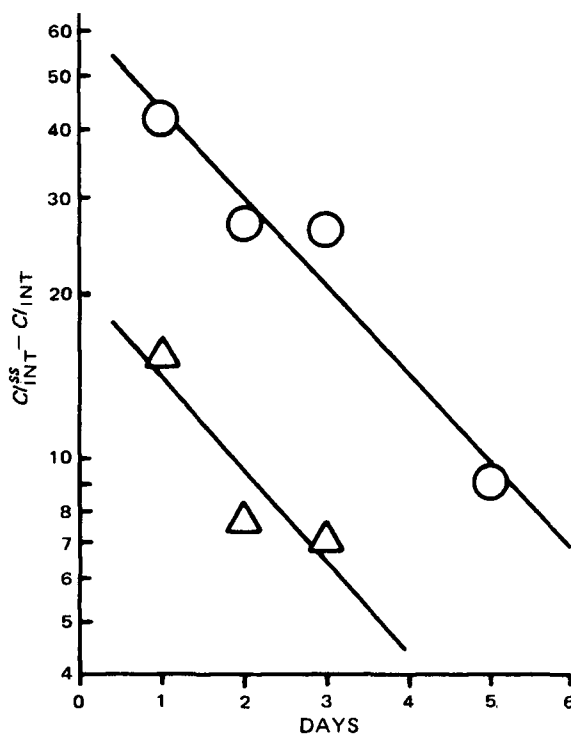

Figure 3—Semilogarithmic plot of Cl_{INT}^{ss} − Cl_{INT} versus time for pentobarbital elimination during the induction phase (0–10 days). Key: △, data for the 25-ppm pretreatment, and ○, data for the 125-ppm pretreatment.

clearances, which are more realistic indexes of the degree of induction. This is especially true if the probe molecule under control conditions undergoes a facile liver metabolic clearance that borders on liver blood flow rate dependency.

The lack of significant short-term effects of the 1- and 5-ppm pretreatments on pentobarbital pharmacokinetics suggests that a critical mass of inducer is needed to initiate liver microsomal enzyme stimulation. At the 25-ppm level, an increase in the pentobarbital intrinsic clearance was seen only on Day 5 of exposure, whereas a significant increase in the intrinsic clearance was seen on the 1st day of exposure at the 125-ppm level. These results are consistent with the need for a critical mass of inducer to accumulate before induction is initiated.

Levy and coworkers (45–51) recently published several reports dealing with the time profiles of drug induction. An attempt was made to fit the induction time profile seen in the present work by estimating an average steady-state intrinsic clearance, Cl_{INT}^{ss} (38.4 ml/min/kg for 25 ppm and 80.8 ml/min/kg for 125 ppm), and plotting log ($Cl_{INT}^{ss} - Cl_{INT}$) versus time for the induction phase. Good first-order plots were obtained (Fig. 3), giving apparent induction half-lives of ~2 days for both the 25- and 125-ppm data. Similarly, postinduction data were treated by plotting log ($Cl_{INT} - Cl_{INT}^{0}$) versus time, where Cl_{INT}^{0} is the mean value of the intrinsic clearance for the control animals. Although half-lives for the loss of residual induction effects of ~11 days were obtained for both the 25- and 125-ppm data, care must be taken in interpreting these values because of the limited number of points available in the postinduction phase.

Based on the observations in this study, the following tentative conclusions can be made about polychlorinated biphenyl induction of pentobarbital elimination.

1. Induction by the polychlorinated biphenyls shows an apparent lag time that appears to be dose dependent.

2. The kinetics of induction, once initiated, appear to be first order, and the induction half-life seems to be dose independent.

3. The extent of induction is polychlorinated biphenyl dose dependent, but there is an apparent minimum exposure below which significant induction is not seen.

4. After removal of the polychlorinated biphenyls, there appears to be a lag time before the various induced pharmacokinetic parameters begin to return to control values (Figs. 1–3).

5. An apparent half-life for the loss of residual induction effects seems to be dose independent.

Although these conclusions must be tempered because of the limited number of data points and doses studied, it is interesting to speculate on their significance. If relationships such as those implied here can be substantiated, they may allow prediction of the time course of induction by various levels and times of exposure of enzyme-inducing agents such as polychlorinated biphenyls and of the effect of this induction on the pharmacokinetics of drugs such as pentobarbital. A better understanding of this phenomenon will allow more quantitative prediction of the therapeutic consequences of accidental acute or chronic exposure to agents such as polychlorinated biphenyls and other environmental enzyme inducers.

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