

Toxicokinetic and Toxicodynamic Influences on Endocrine Disruption by Polychlorinated Biphenyls

Sermpan Soontornchat, Mei-Hui Li, Paul S. Cooke, and Larry G. Hansen

Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61801 USA

Polychlorinated biphenyl (PCB) mixtures and individual chlorobiphenyl (CB) congeners have various endocrine-disrupting effects, but ultimate responses may be altered by concurrent effects on enzyme levels and enzyme activities. The toxicodynamics of estrogenic PCBs and metabolites have been studied *in vitro*, but nonlinear dose-response relationships *in vivo* suggest that tests must integrate toxicokinetic parameters to explain whole-animal responses. To determine if any such interactions occurred, relatively large doses were subdivided into different treatment regimens for immature female Sprague-Dawley rats. Aroclor 1242 was uterotrophic when 120 mg/kg (total) was administered (intraperitoneally) in two, three or five doses. CB 47 (2,2',4,4'-tetraCB) and CB 153 (2,2',4,4',5,5'-hexaCB) increased absolute uterine weights at 30 mg/kg on days 20 and 21. Results at 25 days in rats that received zero, two, three or five doses between days 20 and 24 were much more variable due to changes in tissue responsiveness and/or toxicokinetic interactions. In rats receiving treatment for 5 days, pentoxoresorufin *O*-dealkylase (PROD) activity was inversely related to CB serum residues; in rats receiving CB 153 for 2 days, PROD activity was directly related to serum residues. It was not clear whether PROD activity was the cause of or a reflection of lower serum residues; however, nonplanar CBs are better substrates for PROD than are planar CBs, and the longer-term dosing may enhance metabolism and excretion, changing the biological effects observed. **Key words:** chlorobiphenyl, estrogenicity, pentoxoresorufin *O*-dealkylase, polychlorinated biphenyl, toxicodynamics, toxicokinetics. *Environ Health Perspect* 102:568-571 (1994)

The endocrine-disrupting actions of chlorinated aromatic chemicals are well known, and concern has recently heightened because of their ubiquitous presence in the environment and in food (1). Toxicodynamics, i.e., agonist-receptor interactions (2), play a critical role in endocrine disruption; however, particularly for xenobiotics, which are enzyme inducers and/or yield bioactive metabolites, toxicokinetics play an equally critical role. Neither residues of parent xenobiotic in the target tissue nor affinity for the target receptor alone can reliably predict toxicity. Integrated toxicodynamic and toxicokinetic considerations are essential to meaningful risk assessment.

Polychlorinated biphenyls (PCBs) are persistent global pollutants with a net estrogenic activity in most mixtures (3-5),

even though a few of the 209 possible chlorobiphenyl (CB) congeners, namely, the coplanar Ah receptor agonists, are antiestrogenic under some conditions (5,6). The biological activities of Ah receptor agonists can generally be predicted based on their induction of hepatic microsomal ethoxyresorufin *O*-dealkylase (EROD; P4501A1) as compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent Ah receptor agonist (7). The environmentally more abundant *ortho*-chlorinated congeners are nonplanar and generally poor ligands for the Ah receptor. Di- and tri-*ortho*-chlorinated congeners generally show a phenobarbital-type pattern of monooxygenase induction and induction potencies can be compared by measuring pentoxoresorufin *O*-dealkylase (PROD; P4502B1) (7). Some mono-*ortho* CBs are "mixed" inducers, causing elevation of both EROD and PROD activities (7). Phase 2 conjugating enzymes are also differentially induced.

Microsomal monooxygenases and phase 2 conjugating enzymes play a pivotal role in PCB toxicoses. Not only are these enzymes differentially induced by different CB congener types, but the CB congeners as well as endogenous hormones are differentially metabolized by the various isozymes (8). Some hydroxy CB metabolites may have enhanced uterotrophic potency (3,9). At the same time, other metabolites may inhibit P450 activity *in vitro* (10) or depress P450 activity *in vivo* (11,12), probably through downregulation of synthesis. The methyl sulfonyl metabolites (which are not generated in tissue culture systems) can also induce P450s (12), but they may inhibit the activity of TCDD-induced P450s in human cell lines (13).

Therefore, subtle differences in toxicokinetics may be impossible to extrapolate from independent structure-activity relationships, and the net effect of different chemicals on endocrine functions may be even more elusive. The demonstration that two consecutive doses of a specific CB or PCB mixture are more effective than a single larger total dose (5) raises the possibility that the initial dose may induce monooxygenases that enhance the bioactivation of the second dose to a more potent estrogen. On the other hand, if the initial CB merely acts as a priming dose (as with estradiol), the induction of monooxygenases and, especially phase 2 conjugating enzymes, would be expected to attenuate the estrogenic action.

Parallel assessment of estrogenicity and monooxygenase induction might suggest further interactions between biotransformation and endocrine disruption. The prepubertal rat is a sensitive model for estrogenicity if short-term studies, completed before puberty, are required. Acute responses were considered more useful for determining primary actions and interactions because final toxic outcomes after longer dosing and/or observation periods are complicated by endocrine responses to disruption, other repair responses, progression of initial changes to other pathologies, and more complicated toxicokinetics. The sensitivity of the model to estrogenic PCBs is enhanced by dividing the dose over 2 days (5), but it was unclear how further modifications between weaning and puberty might affect the sensitivity; furthermore, integration of enzyme induction and other toxicokinetic factors into the model required additional time-response considerations.

Materials and Methods

We selected two persistent nonplanar congeners that have different potencies for inducing P4502B1. CB 153 (2,2',4,4',5,5'-hexaCB), recently reported to be estrogenic in the divided-dose protocol (14), and CB 47 (2,2',4,4'-tetraCB), which was reported to have little estrogenic activity in the single-dose protocol (15), were compared in 2-day and 5-day dosing regimens. Aroclor 1242, a moderate mixed inducer of monooxygenases (7,14), is estrogenic in immature rats in both single-dose and double-dose protocols (2-4;14,15); we used this mixture as a reference toxicant to determine the effect of the 5-day dosing protocol on uterotrophic and monooxygenase induction activities.

Female Sprague-Dawley rats nursing litters of 9-11 female pups were purchased from Harlan Sprague-Dawley (Indianapolis, Indiana) and shipped when the litters were 10-15 days of age. At 20 days of age, the pups were weaned, weighed, and separated into dosage groups divided as evenly as possible among litters. Pups were dosed intraperitoneally with PCBs in 0.1 ml corn

Address correspondence to L.G. Hansen, Department of Veterinary Biosciences, University of Illinois, 2001 S. Lincoln Avenue, Urbana, IL 61801 USA.

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oil. A positive control (1 µg estradiol in 0.1 ml corn oil) was included from most litters, and a negative control (corn oil alone) was included from all litters. In the 2-day protocol, we dosed pups on days 20 and 21 of age and killed them on day 22; a limited experiment postponed weaning until day 21, and the rats were killed on day 23. In the 5-day protocol pups were dosed on days 23 and 24, days 20, 22, and 24, or days 20, 21, 22, 23, and 24 and killed on day 25. Positive and negative controls received the three-dose treatment protocol.

One day after the last dose, we weighed pups, decapitated them and collected blood from the cervical stump. The reproductive tract was removed and the uterus trimmed of adhering fat and connective tissue and weighed. The liver was perfused *in situ* with ice-cold Tris-KCl (0.05 M Tris-HCl at pH 7.4 + 0.15 M KCl), removed, weighed, divided into two equal portions and homogenized by hand in Tris-KCl. We resuspended microsomes prepared from the 10–12% homogenates and stored them at -80°C until further dilution and assay for protein, EROD, and PROD (14). For rats killed on day 25, the uteri were homogenized by hand in 1.0 ml Tris buffer and analyzed for protein content.

Blood was allowed to clot, and serum was decanted and stored at -20°C until analyzed for PCB residues by hexane-acetone extraction, sodium sulfate dehydration, and GLC (14). Recoveries from seeded samples were 100.24% ± 0.01% for CB 47 and 98.78% ± 1.30% for CB 153. For some animals, we also removed 100 µl of the liver homogenate and stored it at -20°C for PCB analysis (recovery = 96.3% ± 4.2%).

Results

Estradiol clearly increased uterine weights and protein content in prepubertal female rats whether administered on days 20 and 21, or days 20, 22, and 24 (Table 1). A total dose of 120 mg/kg Aroclor 1242 significantly increased absolute uterus weights and protein content, but relative uterus weights were only marginally different from controls ($p > 0.10$). CB 47 significantly increased uterine weights in the 2-day protocol, and the difference was slightly greater in rats killed on day 23; however, CB 47 was less effective in the more variable rats killed on day 25 in which only uterine protein was significantly increased (Table 1). Two 30 mg/kg doses of CB 153 had a similar effect in 22-day-old rats and 25-day-old rats if two extreme outliers are censored from the 25-day-old rats; however, reducing n to 3 made it difficult to establish significance in the 25-day-old rats (Table 1). A total dose of 50 mg/kg CB 153 divided between days 20 and 21

Table 1. Uterotropic effect of PCBs in immature female rats after various dosing regimens

Treatment (dose × days) ^a	n	Uterine wet weight ^b		Protein (mg/uterus)
		mg	mg/g body weight	
Killed day 22				
Corn oil (0.1 ml × 2)	5	32.8 ± 2.4	0.71 ± 0.06	ND
Estradiol (20 µg/kg × 2)	5	73.8 ± 3.5**	1.47 ± 0.09**	ND
CB 47 (30 mg/kg × 2)	7	47.2 ± 3.0*	0.91 ± 0.05*	ND
CB 153 (30 mg/kg × 2)	7	40.2 ± 2.7 [†]	0.78 ± 0.06	ND
Killed day 23				
Corn oil (0.1 ml × 2)	5	37.6 ± 3.4	0.81 ± 0.06	ND
CB 47 (30 mg/kg × 2)	5	52.9 ± 2.4*	1.16 ± 0.08*	ND
Killed day 25				
Corn oil (0.1 ml × 3)	7	43.1 ± 2.2	0.70 ± 0.03	2.94 ± 0.25
Estradiol (20 µg/kg × 3)	4	60.1 ± 4.2**	0.98 ± 0.04**	4.19 ± 0.51*
CB 47 (30 mg/kg × 5)	6	48.3 ± 3.8	0.71 ± 0.06	3.93 ± 0.42*
CB 153 (12 mg/kg × 5)	5	45.7 ± 4.4	0.71 ± 0.08	3.43 ± 0.28
CB 153 (20 mg/kg × 3)	5	44.3 ± 2.6	0.72 ± 0.04	3.39 ± 0.38
CB 153 (30 mg/kg × 2)	5 ^c	56.7 ± 11.8	0.85 ± 0.15	4.86 ± 0.70*
Aroclor 1242 (24 mg/kg × 5)	5	52.2 ± 3.3*	0.83 ± 0.06	4.33 ± 0.36*
Aroclor 1242 (40 mg/kg × 3)	5	49.1 ± 2.2*	0.83 ± 0.05	4.62 ± 0.16*
Aroclor 1242 (60 mg/kg × 2)	5	50.8 ± 2.7*	0.84 ± 0.04	3.92 ± 0.24*

ND, not determined.

^aDoses administered intraperitoneally either 2 successive days before termination, 3 alternate days before termination, or 5 successive days before termination.

^bValues are means ± SEM. Data were analyzed by Dunnett's test for multiple comparison in three separate data sets as determined by age at termination. (*, **, †) Significant ($p < 0.05$) difference from corn oil controls in each data set; different symbols indicate significant differences from other treatment groups in the same data set.

^cThe large variation was improved by censoring two extreme data points. For $n = 3$, significant ($p < 0.05$) increases in uterine weight (48.5 ± 1.6) and uterine protein (4.34 ± 0.07) were demonstrated, but the increase in relative uterine weight (0.076 ± 0.02) was not significant with the lower n .

Table 2. Liver microsomal protein and ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD) activity in immature female rats receiving PCBs in various dosing regimens

Treatment (dose × days) ^a	n	Liver (% body)	Protein ^b (mg/g liver)	pmol/min/mg protein ^b	
				EROD	PROD
Killed day 22					
Corn oil (0.1 ml × 2)	5	4.7 ± 0.2	8.6 ± 0.9	184 ± 40	7.8 ± 1.3
Estradiol (20 µg/kg × 2)	4	4.6 ± 0.3	10.7 ± 1.5*	169 ± 35	6.6 ± 1.5
CB 47 (30 mg/kg × 2)	5	5.1 ± 0.2	11.4 ± 1.3*	136 ± 29	8.4 ± 1.9
CB 153 (30 mg/kg × 2)	5	4.9 ± 0.2	12.2 ± 1.6*	161 ± 37	14.0 ± 3.2*
Killed day 23					
Corn oil (0.1 ml × 2)	5	4.6 ± 0.1	13.9 ± 1.4	167 ± 17	8.2 ± 0.7
CB 47 (30 mg/kg × 2)	5	4.9 ± 0.1	14.3 ± 1.1	312 ± 28*	17.7 ± 1.7*
Killed day 25					
Corn oil (0.1 ml × 3)	6	5.3 ± 0.1	11.1 ± 0.4	95 ± 1	4.7 ± 0.3
Estradiol (20 µg/kg × 3)	4	5.2 ± 0.1	12.3 ± 1.5	108 ± 7	6.5 ± 1.0
CB 47 (30 mg/kg × 5)	4	5.9 ± 0.2*	16.6 ± 2.0*	90 ± 4	23.8 ± 5.7*
CB 153 (12 mg/kg × 5)	5	5.7 ± 0.1*	16.4 ± 1.6*	120 ± 8	32.5 ± 5.7*
CB 153 (20 mg/kg × 3)	5	5.8 ± 0.2*	15.0 ± 1.5*	120 ± 6	33.0 ± 6.8*
CB 153 (30 mg/kg × 2)	5	5.4 ± 0.1	16.0 ± 1.7*	110 ± 14	35.0 ± 10.8*
Aroclor 1242 (24 mg/kg × 5)	3	5.5 ± 0.1*	15.0 ± 0.2*	883 ± 77*	7.4 ± 0.6 [†]
Aroclor 1242 (40 mg/kg × 3)	3	5.8 ± 0.2*	14.6 ± 0.7*	949 ± 93*	9.5 ± 0.6 [†]
Aroclor 1242 (60 mg/kg × 2)	3	5.5 ± 0.1*	13.3 ± 1.4	1072 ± 116*	9.5 ± 1.0 [†]

^aDoses were administered intraperitoneally either 2 successive days before termination, 3 alternate days before termination, or 5 successive days before termination.

^bValues are means ± SEM. (*, †) Significant ($p < 0.05$) differences from corn oil controls in each data set (as determined by termination age); different symbols indicate significant differences from other treatment groups in the same data set.

has previously been shown to produce a peak uterotrophic effect; declining effect was associated with increased PROD activity at 100 mg/kg, and both effect and PROD activity were lower at 120 mg/kg (14). The high variability for CB 153 could be partly due to apparent steep time-response and dose-response U-shaped relationships

influenced by toxicokinetics and must be examined more closely.

Induction of hepatic EROD activity was not apparent in rats treated with either CB except for a slight increase in 23-day-old rats after CB 47 treatment (Table 2). Aroclor 1242 increased EROD activity 9- to 10-fold in 25-day-old rats, regardless of

the schedule for delivering the total dose of 120 mg/kg.

Again, the division of the total dose over 5 days did not significantly influence induction of PROD by CB 153 or Aroclor 1242; PROD activity was induced about five-fold by a total dose of 150 mg/kg CB 47 or 60 mg/kg CB 153, but only about two-fold by 120 mg/kg Aroclor 1242 (Table 2). Induction of PROD activity was less pronounced in slightly younger rats. CB 153 only doubled PROD specific activity when administered at the same dose on days 20 and 21. PROD activity was not changed by 30 mg/kg CB 47 on days 20 and 21, but was doubled when dosing was delayed to days 21 and 22 (Table 2).

Residues of the two persistent CBs in the serum were high (up to 22 µg/ml) 1 day after the last of multiple intraperitoneal doses (Table 3). CB 47 residues were equal the day after two intraperitoneal doses of 30 mg/kg whether the doses were administered on days 20 and 21 or days 21 and 22 (Table 3). The serum residues after five daily doses of 30 mg/kg CB 47 were twice that of residues after two daily doses and more variable. Residues were higher in rats with lower total PROD activity (specific activity × mg protein/g liver × g liver) than in rats with greater than twofold higher PROD activity (Fig. 1). This relationship among individual rats was not apparent after two daily doses.

As with CB 47, five daily doses of CB 153 resulted in higher and more variable serum residues than two or three higher doses (Table 3). Greater total PROD activity also tended to be associated with lower PCB residues in rats given five daily doses of 12 mg/kg CB 153, but the relationship was not as clear as with CB 47 (Fig. 1); moreover, in rats administered the higher

dose on days 23 and 24, the more likely direct relationship between serum CB and PROD activity is seen (Fig. 2). Neither pattern was readily discernable in 22-day-old rats or in 25-day-old rats receiving doses on alternate days; nevertheless, differences in PROD induction contributed to the variability of serum residues or vice versa.

Discussion

CBs 47 and 153 are slowly metabolized mainly by PROD and/or other phenobarbital-induced monooxygenases (8). Small amounts of CB 153 metabolites are transiently (2 hr) present in monkey liver, and much larger amounts persist considerably longer in dog liver (8). The basal biotransformation potential of the rat is between that of the dog (very high) and the monkey (very low) and can be at least doubled by phenobarbital induction (8). During the longer exposure (5 days) in the current study, induced PROD as well as induced phase 2 enzymes should be expected to favor lower serum residues of parent compound; however, the apparent rapid decline of PROD activity toward basal levels after relatively recent declines in parent CB requires further explanation. With highly induced PROD, the concentration of metabolites in the liver would be increased; because free hydroxylated CBs have been shown to inhibit some monooxygenase activities (10), this might explain the more-abrupt-than-expected decrease in measured PROD activity.

In older animals with a larger body fat compartment, CB 153 redistribution to other tissues (rather than metabolism) may be responsible for lower serum residues (16,17) and limit exposure to hepatic enzymes (17); however, in these immature rats with less body fat as well as less time

for equilibria to occur, serum residues are relatively predictive of liver residues (Fig. 3).

The increased inducibility of PROD in 25-day-old rats on the two-dose CB 153 protocol compared to 22-day-old rats introduces greater variability in PROD induction (Table 2). The short-term dosing does not permit stabilization of the high PROD–low serum CB relationship suggested in Figure 1. In fact, in rats dosed on days 23 and 24, variability is great enough to clearly show the more expected direct relationship (Fig. 2). This variability is also reflected in the uterotrophic effect (Table 1), but the greatest increase in uterus weight (to 103 mg) was in the rat with the lowest total PROD activity (and lowest serum parent CB level), whereas the rat with the higher serum residues had a uterus that weighed the same as the lower controls (35 mg). Although closer scrutiny

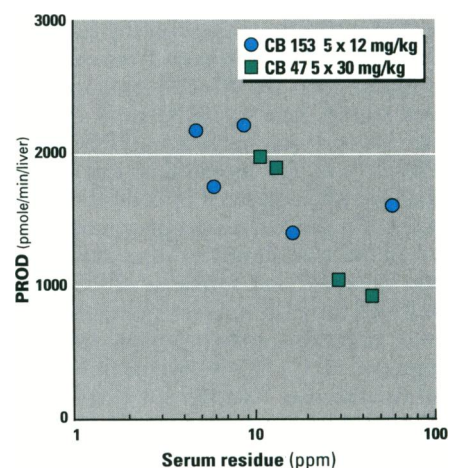


Figure 1. Inverse relationship between pentoxysorufin *O*-dealkylase activity and serum chlorobiphenyl residues in 25-day-old rats dosed with 30 mg/kg CB 47 or 12 mg/kg CB 153 on days 20, 21, 22, 23, and 24.

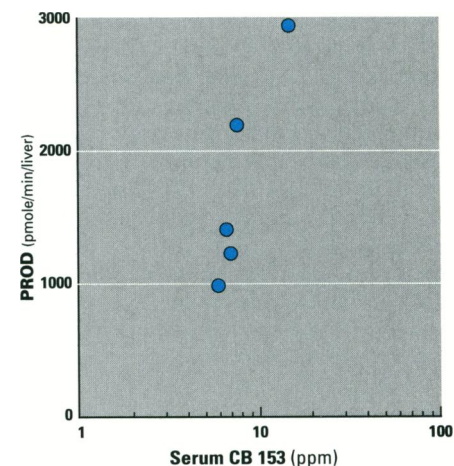


Figure 2. Direct relationship between pentoxysorufin *O*-dealkylase activity and serum chlorobiphenyl 153 residues in 25-day-old rats dosed with 30 mg/kg CB 153 on days 23 and 24.

Table 3. Determination of average actual doses and terminal serum residues in immature female rats receiving PCBs in various dosing regimens

Treatment ^a (dose × days)	n	Actual dose ^b (mg/kg)	Serum residue (µg/ml)
Killed day 22			
CB 47 (30 mg/kg × 2)	4	62.1 ± 1.7	10.94 ± 1.40
CB 153 (12 mg/kg × 2) ^c	4	21.9 ± 0.4	2.97 ± 1.66 ^c
CB 153 (24 mg/kg × 2) ^c	4	49.2 ± 1.4	2.89 ± 2.38 ^c
CB 153 (30 mg/kg × 2)	7	61.5 ± 2.4	11.79 ± 1.68
CB 153 (50 mg/kg × 2) ^c	4	103.0 ± 5.4	13.38 ± 7.50 ^c
CB 153 (60 mg/kg × 2) ^c	4	118.6 ± 5.4	5.22 ± 2.27 ^c
Killed day 23			
CB 47 (30 mg/kg × 2)	5	69.9 ± 3.4	10.79 ± 0.28
Killed day 25			
CB 47 (30 mg/kg × 5)	6	130.9 ± 3.5	22.26 ± 5.43
CB 153 (12 mg/kg × 5)	5	54.9 ± 1.0	18.86 ± 10.08
CB 153 (20 mg/kg × 3)	5	56.7 ± 2.0	7.58 ± 0.99
CB 153 (30 mg/kg × 2)	5	53.7 ± 1.6	8.09 ± 1.62

^aNominal doses administered intraperitoneally either 2 successive days before termination, 3 alternate days before termination, or 5 successive days before termination.

^bActual dose determined by dividing confirmed (by GLC) dose by average weight during dosing period.

^cSASCO Sprague-Dawley rats from previous study (14).

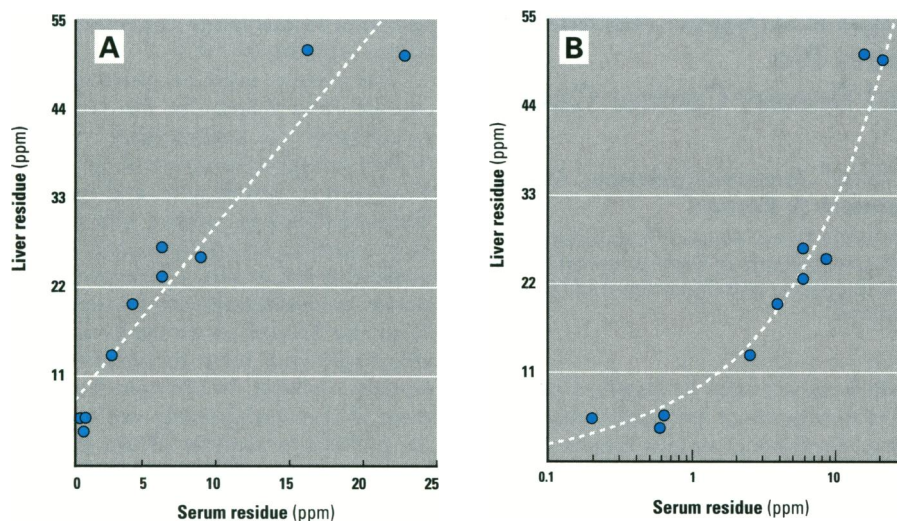


Figure 3. Relationship between serum and liver chlorobiphenyl residues in individual 22-day-old rats given 11, 25, or 51 mg/kg CB 153 on days 20 and 21. The data were fit by (A) linear regression and (B) the exponential equation: $\text{Liver residue} = a_0 e^{(a_1)(\log \text{ serum residue})}$. Correlation coefficients for the data were virtually equal (0.9586 for linear versus 0.9587 for exponential).

with a larger number of animals may be necessary, these large ranges after shorter times in highly induced rats indicate rather steep time–response curves for both toxicokinetics and physiological actions.

Increased uterotrophic potency from divided doses (5) may be due to induced PROD activity and the generation of more bioactive (9) metabolites in the liver (8). Phase 2 enzymes such as uridine diphosphate-glucuronyl transferases (Li M-H, Hansen LG, unpublished results) and glutathione transferases (Gillette J, Dauterman WC, personal communication) were also induced in these rats. Therefore, enhanced phase 2 metabolism competes with enhanced bioactivation in an already complex system so that the increased variability observed should have been expected.

When these relationships are considered collectively, it becomes apparent that developmental differences in toxicokinetics and toxicodynamics interact profoundly with dose selection, frequency, and timing so that narrow windows of response are created. If these whole-animal considerations are not fully explored, inaccurate and misleading conclusions may be reached that will seriously impede effective risk assessment.

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