The Distribution and Excretion of Polychlorinated Biphenyls (Aroclor 1254) and their Effect on Urinary Gonadal Steroid Levels in the Boar

by N. S. PLATONOW, R. M. LIPTRAP, and H. D. GEISSINGER
Department of Biomedical Sciences, Ontario Veterinary College
University of Guelph, Guelph, Ontario, Canada

Risebrough et al.(1) have reported that the environmental contaminants, polychlorinated biphenyls (PCB), have the capacity to enhance steroid-hydroxylating enzyme activity in the liver of the pigeon, thus increasing estradiol metabolism. The organochlorine pesticides of the DDT group exert a similar effect. On a weight basis, the PCB preparation has an estradiol degrading potential of about five times that of p,p'-DDE or technical DDT in liver homogenate preparations. In the rat, Street et al.(2) reported that the sleeping time induced by a standard dose of hexabarbital was reduced as the chlorine content of the various Aroclor preparations increased from 21 to 68 percent. In vitro, these preparations affected the rates of aniline hydroxylation and p-nitroanisole demethylation in a similar manner. Lincer and Peakall(3) reported an increase in the rate of liver metabolism of estradiol in vitro, and increased levels of cytoplasmic RNA, in American kestrels (Falco sparverius) after the feeding of 0.5 and 5 ppm of Aroclor in the diet. The estrogenic activity of various PCB mixtures, evaluated by the glycogen response of the immature rat uterus, has been described by Bitman and Cecil(4).

Recently, Platonow and Funnell(5) reported that the feeding of PCB resulted in anti-androgen effects, as assessed by a decrease in testicular and comb growth, in cockerels. The signs appeared as early as one week after the start of continuous feeding of 250 ppm of PCB in the diet.

Pathological lesions attributed to PCB have been described in the rat(6), Bengalese finch(7), mouse(8), chicken(9,10,11), monkey(8) and man(12). The liver was the organ most affected in mammals, whereas in birds, hydropericardium, kidney damage and reduced spleen size were most commonly observed.

The present study was carried out to determine the effect of oral administration of PCB in the adult boar. In view of the high excretory levels of dehydroepiandrosterone and estrogens by the boar(13), the levels of these testicular steroids was also examined. Since pathological lesions have been described in several species following the ingestion of PCB, an autopsy was performed on each animal at the completion of the experiment.

Methods and Materials

Four Yorkshire boars, approximately two years of age, and weighing 175 to 260 kg were used. One boar (P-10) was given a single dose of 10 mg of PCB per kg of body weight, a second boar (P-100) received a single dose of 100 mg of PCB per kg and the third (P-1x10) and fourth (P-10x10) animals received 10 daily doses consecutively of 1 and 10 mg per kg, respectively. The PCB,

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as Aroclor 1254*, were dissolved in a small volume of olive oil and mixed with the morning ration. The first two animals (P-10, P-100) were euthanized seven days after the single treatment and the other two boars (P-1x10, P-10x10) were euthanized seven days after receiving the last dose. The animals were fed a commercial hog ration twice daily and had access to tap water. During the experiment the animals were confined in a metabolism crate, modified from that described by Welch et al.(14), to facilitate the collection of urine and feces.

Urine and feces were collected once daily. The PCB extraction and subsequent determination by gas chromatography was performed using the chlorinated insecticide method of Saschenbrecker and Ecobichon(15). The quantitation of the PCB was made as described by Armour and Burke(16). Levels of dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) in urine were determined on daily samples using a modified method of Fotherby(17), as described by Raeside(13). The results for total estrogens in the urine samples were estimated with the procedure outlined by Raeside(18).

The animals were euthanized with sodium pentobarbital intravenously and immediately subjected to gross pathological examination. Tissues from selected organs were fixed in 10 percent formol-saline for histological examination. These were embedded in paraffin, cut at 4 to 6 μ on a rotary microtome and stained with hematoxylin and eosin according to routine procedures. Specimens from various organs were removed and stored at -20°C until analyzed. The brain was removed and cut into two exact halves, of which one was homogenized prior to PCB analyses.

Results and Discussion

Tissue Distribution of PCB

The concentrations of PCB in the various tissues are indicated in Table I. The data show that the lowest levels of PCB were present in blood and the highest levels were in perirenal fat. The hepatic tissues contained the second highest level of PCB in the two animals given a single dose. In the two boars receiving multiple doses however, the three muscular tissues contained the second highest amount. In these latter animals also, the levels in the testes were similar to those measured in the liver. The levels of PCB in the kidney of all animals were low, which together with the low urinary levels measured, may indicate a low renal excretion of PCB compounds by the boar.

The ratios between the tissue concentrations for the high and low dose administration were calculated for the single and multiple dose experiments. For animals receiving a single dose, the highest ratio occurred in perirenal fat, 6:1, and the lowest ratio, 1:0.65, was present in the testes. Higher ratios resulted when multiple doses were administered; 11.5:1 in perirenal fat was highest and 1.7:1 in the brain was lowest.

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* Aroclor 1254 was generously supplied by Monsanto Canada Ltd., Toronto, Ontario.

TABLE I

Concentrations (in μg/g wet tissue) of Polychlorinated Biphenyls (Aroclor 1254) in Various Tissues of Boars Given 10 mg/kg (P-10) and 100 mg/kg (P-100) as Single Dose, Respectively, as well as Ten Consecutive Daily Doses of 1 mg/kg (P-1x10) and 10 mg/kg (P-10x10), Respectively.

Tissue	Boar P-10	Boar P-100	Boar P-1x10	Boar P-10x10
Blood	0.3	0.4	0.3	1.1
Brain	2.1	4.2	1.8	3.1
Perirenal Fat	39.8	245.1	13.7	156.9
Kidney	2.9	2.6	0.9	3.4
Liver	6.3	9.3	2.2	6.5
Testicle	5.8	3.8	1.7	5.6
Muscular Diaphragm	4.0	7.8	4.1	18.7
Psoas	3.5	5.6	3.4	11.0
Heart	-	3.5	2.3	6.9

Although animal P-10 and animal P-1x10 received the same total amount of PCB on a body weight basis, considerable variation was observed in the tissue concentrations. The single low dose resulted in higher tissue concentrations than the comparable multiple dose. The reverse occurred however in the two animals given the higher dose level.

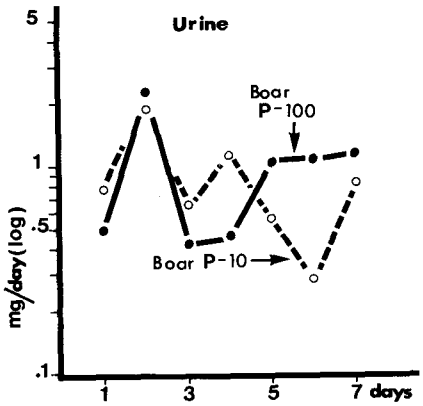
Excretion of PCB

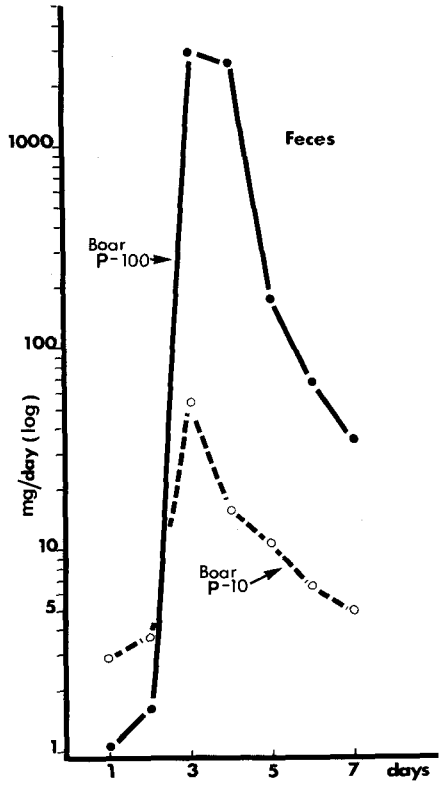
The amounts of PCB excreted in the urine and feces are depicted in Figure 1. The urinary excretion of PCB was substantially lower than that of the feces for all treatment regimes. In boars given a single dose the highest amount measured in urine occurred on the second day, followed by considerable daily variation. Multiple doses resulted in a trend for increasing urinary excretion during the experiment, although considerable daily variation occurred. Following the 10th or 12th day a decline in PCB concentration was noted.

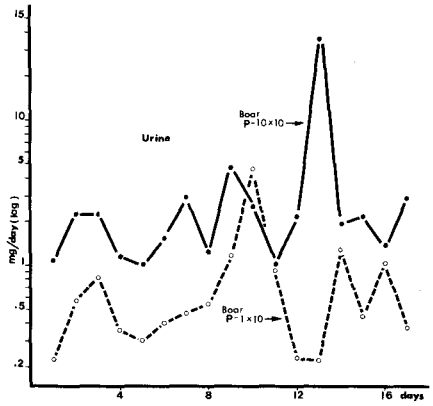
The experimental design used in the present study did not permit the identification of those amounts of PCB which were unabsorbed compared to those portions excreted into the alimentary tract.

The data in Table 2, show the cumulative percentages of the PCB excreted via urine and/or feces. Boars given the low or high single dose of PCB, eliminated in the urine and feces, 12.0 and 16.3 percent respectively of the administered dose during the seven day post-treatment period. For animal P-1x10, 12.7 percent of the total administered dose was accounted for in the urine and feces during and after PCB administration. At the higher, multiple dose level only 6.4 percent was excreted during a similar collection period.

Figure 1.
The amounts of PCB excreted in the urine (upper left and lower left) and feces (upper right and lower right) of boars given high or low doses of PCB orally in single or multiple doses.







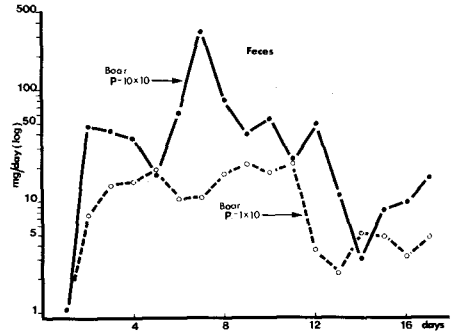


TABLE 2

Cumulative Percentage Excretion of Polychlorinated Biphenyls (Aroclor 1254) in Boars Given 10 mg/kg (P-10) and 100 mg/kg (P-100) as Single Dose, Respectively, as well as Ten Consecutive Daily Doses of 1 mg/kg (P-1x10) and 10 mg/kg (P-10x10) Respectively.

Days	Boar P-10			Boar P-100			Boar P-1x10			Boar P-10x10		
	Urine	Feces	Urine and Feces	Urine	Feces	Urine and Feces	Urine	Feces	Urine and Feces	Urine	Feces	Urine and Feces
1	<0.1	1.5	1.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2	0.1	1.7	1.8	<0.1	<0.1	<0.1	0.2	1.4	1.6	0.1	1.0	1.1
3	0.2	4.4	4.6	<0.1	7.4	7.5	0.3	3.2	3.5	0.1	1.6	1.7
4	0.2	5.2	5.4	<0.1	14.7	14.7	0.3	4.6	4.9	0.1	2.0	2.1
5	0.3	10.7	11.0	<0.1	15.7	15.7	0.4	6.0	6.4	0.2	2.1	2.3
6	0.3	11.0	11.3	<0.1	16.1	16.1	0.4	6.7	7.1	0.2	2.5	2.7
7	0.7	11.3	12.0	<0.1	16.2	16.3	0.4	7.3	7.7	0.2	4.6	4.8
8							0.4	8.1	8.5	0.2	5.1	5.2
9							0.5	9.1	9.5	0.2	5.2	5.4
10							0.7	9.7	10.4	0.2	5.5	5.7
11							0.7	10.6	11.3	0.3	5.6	5.8
12							0.7	10.7	11.4	0.3	5.8	6.0
13							0.7	10.8	11.5	0.4	5.8	6.2
14							1.2	11.0	12.2	0.4	5.8	6.2
15							1.2	11.2	12.4	0.4	5.9	6.3
16							1.3	11.3	12.6	0.4	5.9	6.3
17							1.3	11.5	12.7	0.4	6.0	6.4

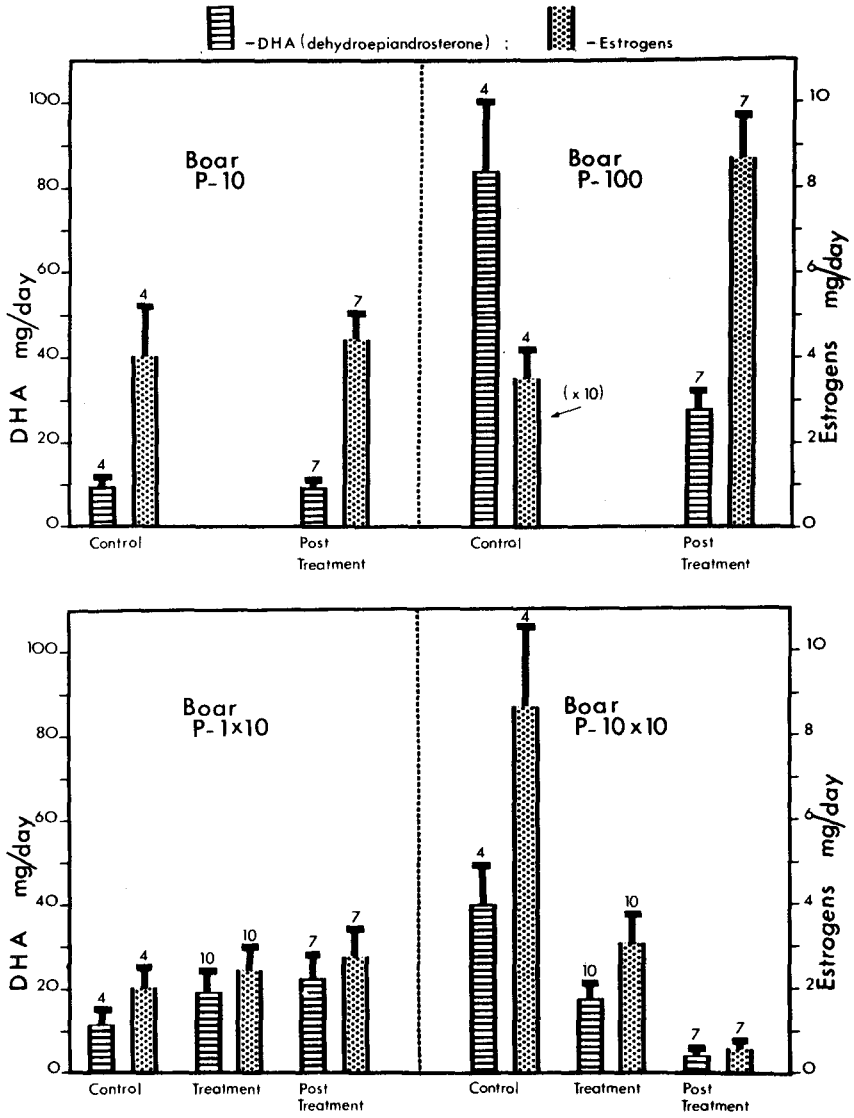


Figure 2.
Urinary excretion of estrogen and dehydroepiandrosterone (DHA) from boars before, during and after the oral administration of single or multiple doses of PCB. Vertical lines above the bar indicate the standard error of the mean. The numbers refer to the number of samples analyzed. Note, for boar P-100 the control value of estrogen shown is only 1/10th of that actually measured.

Excretion of Estrogen and DHA

Figure 2 illustrates the urinary excretion of estrogen and dehydroepiandrosterone (DHA) before, during and after the oral administration of a single or multiple dose of PCB. The lower dosage level for both single and multiple treatments appeared to have no effect upon the urinary excretion of these gonadal steroids. The amounts of these hormones recovered after PCB treatment were not significantly different from those of the control period. However, the urinary levels of these steroids were markedly reduced following ingestion of the higher doses of PCB, in both the single and multiple treatment regimes. With the single high dose, the post-treatment levels or urinary steroids were significantly lower ($P < 0.01$) than control levels in the "t" test, while in the boar receiving the multiple high doses, the steroid levels during both the treatment and post-treatment period were significantly lower ($P < 0.01$) than those of the control period.

Organochlorine compounds such as DDT are known to have an adverse effect upon reproduction in birds, mice, rats and dogs(19). The lower levels of DHA and estrogen excreted in the urine of boars given high doses of PCB suggest that these compounds may also have a deleterious effect upon reproductive activity. The mode of action of PCB in this regard is presently unknown.

Pathology

No macroscopically visible lesions could be seen in any of the experimental animals and these findings were confirmed upon histological examination. The results suggest that although PCB administration to boars may cause significant biochemical alterations, these are not detectable with routine pathological techniques. Lesions of this slight severity may only be detectable with more sophisticated techniques such as electron microscopy.

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