CARDIOVASCULAR AND HEMATOLOGICAL PARAMETERS AFFECTED BY FEEDING VARIOUS POLYCHLORINATED BIPHENYLS TO THE SINGLE COMB WHITE LEGHORN COCKEREL

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The effect of dietary PCBs (50-200 ppm) on some cardiovascular and hematological parameters in the SCWL cockerel was investigated. Heart rate was significantly reduced by PCB 1242 and 1254 at ≥ 100 ppm and was not altered by PCB 1221 or 1260 at 150 ppm. A significant decrease in arterial blood pH was observed with PCB 1254 at 150 ppm. Mean blood pressure was unaffected by any treatment used. Hemoglobin concentration, HCT, and total erythrocyte count were found to be significantly decreased by PCBs 1242 and 1254 at ≥ 50 ppm. These same parameters were not affected by PCB 1221 or 1260 at any level used. It was concluded that the anemia observed was due solely to a decrease in total erythrocyte concentration. The possibility that these changes may be due to a decrease in erythropoiesis is discussed. Pericardial fluid volume was increased with PCBs 1242 and 1254; however, there was no correlation between the magnitude of the bradycardia and the amount of pericardial fluid.

Introduction

Many biological changes have been reported in different animal species after ingestion of polychlorinated biphenyls (PCBs). McCune *et al.* (1962) observed that various levels of Aroclor 1242 produced labored respiration, hydropericardium, enlarged heart, abdominal edema, and a decrease in body weight in chickens. Koeman *et al.* (1969) reported hydropericardium in Japanese quail at 1000 ppm of dietary PCB (60% chlorinated). In addition to the above symptoms, Rehfeld *et al.* (1971) observed internal hemorrhaging, depression of secondary sexual characteristics, and an increase in liver weight as percentage of body weight when PCB (48% chlorinated) was fed to one-day-old chickens. Flick *et al.* (1965) reported that hemoglobin, hematocrit, and blood glucose values were reduced after feeding Aroclor 1242 to White Leghorn cockerels. Little is known concerning the cardiovascular and hematological changes following exposure to various levels of PCB. The following study was undertaken to determine the effects of PCB on some cardiovascular parameters of the White Leghorn cockerel.

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Material and methods

Diets containing different levels of PCBs were prepared by adding to a chick starter ration the respective PCB (Aroclor 1221, 1242, 1254, or 1260; Monsanto Chemical Co., St. Louis, Mo.) (Table I). The PCBs were dissolved in acetone and blended into a premix to facilitate a final even distribution. The acetone was evaporated and the premix blended into the basal ration. An equal amount of acetone-treated premix was added to the basal ration for the control groups. The chicks were reared in starting batteries with raised wire floors, weighed, individually wing banded, and randomly distributed into groups of 12 birds. Feed and water were available *ad libitum*. Weekly feed consumption was measured. All parameters studied were measured for each bird. Systemic arterial blood pressure and heart rate were recorded on a Grass polygraph (Model 7) through a cannulated carotid artery using a Statham pressure transducer (Model P23). Mean blood pressure was calculated according to Sturkie (1965): mean blood pressure = 3/8 pulse pressure + diastolic blood pressure. Blood samples for analysis were taken in heparinized syringes from the cannulated carotid artery. Packed erythrocyte volume (HCT) was determined by collecting two samples in heparinized capillary tubes. The tubes were centrifuged at 11,500 rpm for five minutes in an International microhematocrit centrifuge. Total erythrocyte counts were made by diluting blood 1:200 with Wiseman's solution in dilution pipettes and counting in a hemocytometer (Exp. 1) or by diluting 20λ of blood 1:50,000 with Isoton solution for counting on a Coulter Counter (Model B) (Exp. 2). The mean of two determinations was used as an observation.

Table I. *Chick Starter Ration*

Ingredient	Pounds per ton of feed
Yellow corn, fine ground	1060
Ground oats	100
Wheat middlings	100
Alfalfa meal, dehydrated, 17% protein	80
Meat and bone scraps, 50% protein	50
Solvent process soybean meal, 45% protein	500
Fish meal, Vitaproil, 55% protein	40
Dried whey	40
Ground limestone	10
Dicalcium phosphate (24% Ca, 18.5% P)	10
Salt, iodized	6
Vitamin trace mineral premix	5
Coccidiostat	+
	2001 +

Mean corpuscular volume (MCV) was determined by dividing the packed cell volume by the total erythrocyte count (Wintrobe 1961). Volumes were converted to cubic microns (μ^3) using appropriate conversion factors.

Hemoglobin was determined using the modified Newcomer acid hematin method (Denington and Lucas 1955). Standard curves were made using a prediction equation determined by linear regression using four hemin standards (5, 7.5, 10, and 15 $\times 10^{-3}$ mg/ml). These hemin standards corresponded to 5.05, 7.58, 10.11, and 15.16 gm of hemoglobin per 100 ml of blood, respectively, as determined from a commercial hemoglobin standard (Hycel Hemoglobin control; Hycel, Inc., Houston, Texas). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing hemoglobin concentration by HCT. Blood samples for pH measurement (Corning, Model 12 pH meter and Beckman thermomatic constant temperature block), in experiment 2 were obtained from the cannulated carotid artery. Hydropericardium volume was determined by inserting a 20-gauge needle attached to a 2.5-ml syringe into the pericardium and withdrawing the fluid measured to the nearest 0.1 ml.

Data were analyzed by either analysis of variance or row by column chi-square analysis. Parameters showing significant effects by analysis of variance were further tested by the new multiple range procedure of Duncan (1955).

Experiment 1. In trial 1, rations containing 25, 50, and 100 ppm of Aroclor 1242 were fed to one-day-old pure strain Single Comb White Leghorn (SCWL) cockerels maintained on our research farm. In a second trial the same Aroclor was added to rations to give 50, 100, and 200 ppm. All groups were terminated at eight weeks except in trial 2, when the group fed 200 ppm was sacrificed at four weeks due to high mortality. A corresponding number of controls were sacrificed at this time.

Experiment 2. Rations containing 50, 100, and 150 ppm of Aroclor 1221, 1254, and 1260 were given to one-day-old commercial (DeKalb strain) SCWL cockerels. The experiment was terminated at nine weeks of age.

Results and discussion

The effects on heart rate and mean blood pressure of experiments 1 and 2 are shown in Tables II and III. A significant decrease in heart rate was observed when Aroclor 1242 was present in the diet at 100 or 200 ppm, and when Aroclor 1254 was added in the diet at 100 or 150 ppm. No effect on heart rate was observed for Aroclors 1221 and 1260 at the levels fed. There was no change in mean blood pressure with any PCB at the levels fed. It is known that cholinesterase activity is decreased with lowered pH (Hestrin 1950); hence in experiment 2 arterial blood pH was measured in an attempt to correlate this parameter with the possible change in heart rate as observed with Aroclor 1242 feeding. Using this fact, Emerson *et al.* (1964) postulated that the bradycardia observed in dogs treated acutely with endrin was the result of increased acetylcholine activity. Our results were inconclusive because arterial blood pH was lowered only when the cockerels were fed 150 ppm (Aroclor 1254) whereas bradycardia was also observed at 100 ppm with no change in arterial blood pH (Table III).

Table II. *The Effect of Feeding Rations Containing Aroclor 1242 on Heart Rate and Mean Blood Pressure of SCWL Cockerels*

Parameter	Length of feeding period (weeks)	Dietary levels (ppm)				
		0	25	50	100	200
Heart rate (beats/min)	8 (trial 1)	(12) 398 ± 8.8^{ab}_{m}	(12) 371 ± 10.9_{mn}	(11) 361 ± 10.9_{n}	(7) 349 ± 15.5_{n}	–
	8 (trial 2)	(10) 388 ± 8.9_{x}	–	(12) 351 ± 12.5_{xy}	(10) 328 ± 15.3_{y}	–
	4 (trial 2)	(6) 436 ± 13.2_{x}	–	–	–	(6) 326 ± 23.1_{y}
Mean blood pressure (mm Hg)	8 (trial 1)	(12) 137 ± 4.2	(12) 142 ± 2.7	(11) 142 ± 4.6	(7) 149 ± 7.3	–
	8 (trial 2)	(10) 133 ± 3.7	–	(12) 136 ± 6.9	(10) 128 ± 4.8	–
	4 (trial 2)	(6) 133 ± 7.9	–	–	–	(6) 126 ± 8.6

[a](No. of birds) mean ± standard error.

[b]Mean having different subscripts are significantly different; m, n ($P < 0.05$); x, y ($P < 0.01$).

Table III. *The Effect of Feeding Rations Containing Aroclors 1221, 1254, and 1260 on Heart Rate, Mean Blood Pressure, and Arterial Blood pH of SCWL Cockerels*

Parameter	Aroclor	Dietary levels (ppm)			
		0	50	100	150
Heart rate (beats/min)	1221	(11) 380 ± 7.9[a]	(11) 380 ± 7.2	(10) 390 ± 11.8	(11) 390 ± 8.0
	1254	(12) 398 ± 8.3_x[b]	(12) 372 ± 14.0_{xy}	(8) 343 ± 16.5_y	(7) 328 ± 14.0_y
	1260	(11) 388 ± 14.9	(12) 380 ± 8.7	(12) 366 ± 9.2	(12) 381 ± 10.3
Mean blood pressure (mm Hg)	1221	(12) 150 ± 4.1	(12) 145 ± 9.9	(12) 149 ± 3.8	(12) 148 ± 8.9
	1254	(12) 148 ± 3.2	(12) 148 ± 4.0	(8) 145 ± 10.0	(7) 125 ± 10.4
	1260	(11) 139 ± 4.1	(11) 147 ± 3.8	(10) 148 ± 5.7	(10) 142 ± 3.8
Arterial blood pH	1221	(11) 7.66 ± 0.011	(11) 7.63 ± 0.013	(10) 7.65 ± 0.015	(11) 7.68 ± 0.009
	1254	(12) 7.65 ± 0.010_m	(11) 7.65 ± 0.014_m	(8) 7.66 ± 0.023_m	(7) 7.59 ± 0.017_n
	1260	(12) 7.67 ± 0.015	(12) 7.65 ± 0.011	(10) 7.67 ± 0.022	(12) 7.66 ± 0.011

[a](No. of birds) mean ± standard error.

[b]Means having different subscripts are significantly different; m, n ($P < 0.05$); x, y ($P < 0.01$).

The effects of Aroclor 1242 on HCT, hemoglobin concentration, total erythrocyte concentration, MCV, and MCHC are presented in Tables IV and V. It is evident that HCT, hemoglobin concentration, and total erythrocyte concentration were significantly reduced by Aroclor 1242. Tables VI and VII show the effect of Aroclors 1221, 1254, and 1260 on the same parameters. Aroclor 1254 at levels as low as 50 ppm in the diet caused a significant decrease in HCT, hemoglobin concentration, and total erythrocyte concentration, whereas neither Aroclor 1221 nor 1260 had any effect on these parameters at the dietary levels fed. MCV and MCHC were not significantly altered. Similar changes in HCT and hemoglobin concentration have been reported by Flick *et al.* (1965) as early as one week of age after feeding Aroclor 1242 at 200 and 400 ppm in the diet. They believed that these changes were transient since recovery was observed at three weeks of age. However, the experiment was terminated at that time and no further observations were made. Rehfeld *et al.* (1972a, b) observed decreases in hemoglobin concentration and HCT when feeding Aroclor 1248 at levels as low as 20 ppm and 30 ppm for four and one-half weeks. These changes were reversible when the PCB was withdrawn after two and one-half weeks.

The anemia observed when feeding PCBs to cockerels could be due to a decrease in total concentration of erythrocytes, a decrease in erythrocyte size, an increase in plasma volume, a decrease in mean corpuscular hemoblobin concentration, or any combination of the above. An increase in plasma volume is improbable since loss in body weight and edema formation was observed with Aroclor 1242 at 50 ppm and Aroclor 1254 at 100 ppm. Edema formation has been reported by other investigators when feeding various PCBs (McCune *et al.* 1962, Flick *et al.* 1965, De Vos and Koeman 1970, Rehfeld *et al.* 1971). Since there were no significant differences in MCV and MCHC, it was concluded that the normochromic normocytic anemia was due solely to a decrease in total erythrocyte concentration. This change may be explained by a decrease in erythropoiesis which is partially controlled by sex steroids. A marked decrease in comb size, indicating reduced testosterone secretion, was observed in birds fed Aroclors 1242 and 1254 although it was not quantitated. This observation is in agreement with other workers (Rehfeld *et al.* 1971, Platonow and Funnell 1971). Several investigators have reported that testosterone increases total erythrocyte concentration in chickens (Domm *et al.* 1943, Domm and Taber 1946, Tanaka and Rosenberg 1955) and in Japanese quail (Nirmalan and Robinson 1972). Also, Fried *et al.* (1966), Alexanian *et al.* (1967), and Malgor and Fisher (1970) have suggested that testosterone has an effect on erythropoiesis by stimulating *in vitro* production of erythropoietin in mammalian species.

PCB may decrease red cell formation through enhanced hepatic catabolism of testosterone (Nowicki and Norman 1972). Another possibility is that PCB's may directly affect erythropoiesis or erythropoietin production. This is supported by the work of Bitman *et al.* (1972) who demonstrated that some PCBs have estrogenic-like activity in rats. It has been suggested that estrogens depress erythropoietin precursor formation.

There was a significant relationship between dietary level of PCB's and hydropericardium (Table VIII); however, there was no correlation within individual treatments between the magnitude of the bradycardia and the amount of pericardial fluid.

Table IV. *The Effect of Feeding Rations Containing Aroclor 1242 (Trial 1) on the Packed Cell Volume, Hemoglobin Concentration, Total Erythrocyte Count, Mean Corpuscular Volume, and Mean Corpuscular Hemoglobin Concentration of SCWL Cockerels*

Parameter	Length of feeding period	Dietary levels (ppm)			
		0	25	50	100
Packed cell volume (%)	8 wks	(12) 27.4 ± .83$_x$[a,b]	(12) 25.8 ± .59$_{xy}$	(10) 23.3 ± .86$_y$	(7) 19.8 ± .31$_z$
Hemoglobin conc. (gm/100 ml)	8 wks	(12) 9.24 ± .316$_x$	(12) 8.39 ± .180$_y$	(11) 7.59 ± .205$_y$	(7) 6.29 ± .135$_z$
Total erythrocyte count (millions/mm^3)	8 wks	(12) 2.77 ± .134$_x$	(11) 2.53 ± .121$_x$	(10) 2.56 ± .096$_x$	(7) 2.00 ± .168$_y$
Mean corpuscular volume (μ^3)	8 wks	(11) 96.1 ± 2.46	(10) 99.2 ± 3.09	(9) 93.9 ± 3.10	(7) 99.7 ± 2.51
Mean corpuscular hemoglobin conc. (gm/100 ml)	8 wks	(12) 33.8 ± .53	(12) 32.6 ± .53	(10) 32.7 ± .68	(7) 31.8 ± .34

[a](No. of birds) mean ± standard error

[b]Means having different subscripts are significantly different; x, y, z ($P < 0.01$).

Table V. *The Effect of Feeding Rations Containing Aroclor 1242 (Trial 2) on the Packed Cell Volume, Hemoglobin Concentration, Total Erythrocyte Count, Mean Corpuscular Volume, and Mean Corpuscular Hemoglobin Concentration of SCWL Cockerels*

Parameter	Length of feeding period	Dietary levels (ppm)			
		0	50	100	200
Packed cell volume (%)	8 wks	(10) 23.4 ± 0.74^{ab}_{m}	(12) 20.8 ± 1.56_{mn}	(10) 17.3 ± 1.53_{n}	–
	4 wks	(6) 25.1 ± 1.40	–	–	(6) 20.6 ± 2.69
Hemoglobin conc. (gm/100 ml)	8 wks	(10) 8.28 ± $.351_{x}$	(12) 7.47 ± $.354_{xy}$	(10) 5.86 ± $.638_{y}$	–
	4 wks	(6) 7.51 ± .270	–	–	(6) 6.24 ± 1.025
Total erythrocyte count (millions/mm^3)	8 wks	(10) 2.64 ± $.089_{m}$	(12) 2.24 ± $.170_{mn}$	(10) 1.91 ± $.192_{n}$	–
	4 wks	(6) 2.61 ± .053	–	–	(6) 2.15 ± .300
Mean corpuscular volume (μ^3)	8 wks	(10) 88.8 ± 1.83	(12) 93.8 ± 1.81	(10) 92.3 ± 2.56	–
	4 wks	(6) 96.0 ± 3.92	–	–	(6) 96.8 ± 2.07
Mean corpuscular hemoglobin conc. (gm/100 ml)	8 wks	(10) 35.2 ± 0.68	(11) 34.3 ± 0.62	(10) 33.8 ± 0.95	–
	4 wks	(6) 30.1 ± 0.95	–	–	(5) 31.2 ± 0.37

[a](No. of birds) mean ± standard error.

[b]Mean having different subscripts are significantly different; m, n ($P < 0.05$); x, y ($P < 0.01$).

Table VI. *The Effect of Feeding Rations Containing Aroclors 1221, 1254, and 1260 on the Packed Erythrocyte Volume, Total Erythrocyte Count, and Hemoglobin Concentration of SCWL Cockerels*

Parameter	Aroclor	Dietary levels (ppm) 0	50	100	150
Packed erythrocyte volume (%)	1221	(11) 29.5 ± .45[a]	(11) 27.4 ± 1.46	(10) 29.5 ± .57	(11) 26.7 ± .91
	1254	(12) 29.5 ± $.44_x^b$	(10) 23.3 ± 1.36_y	(8) 20.4 ± 2.36_y	(7) 18.0 ± 1.86_y
	1260	(12) 27.6 ± .96	(12) 28.8 ± .79	(12) 27.4 ± .89	(12) 26.6 ± .61
Total erythrocyte count (millions/mm^3)	1221	(11) 2.25 ± .061	(11) 2.04 ± .096	(10) 2.22 ± .103	(11) 2.12 ± .157
	1254	(12) 2.16 ± $.074_m$	(10) 1.84 ± $.119_n$	(7) 1.77 ± $.168_n$	(6) 1.48 ± $.118_n$
	1260	(11) 2.08 ± .096	(12) 2.16 ± .077	(12) 1.96 ± .063	(12) 1.96 ± .063
Hemoglobin conc. (gm/100 ml)	1221	(11) 9.87 ± .156	(11) 9.04 ± .565	(10) 9.91 ± .256	(11) 9.03 ± .293
	1254	(12) 10.04 ± $.182_x$	(11) 7.91 ± $.416_y$	(8) 6.97 ± $.906_y$	(7) 6.10 ± $.728_y$
	1260	(11) 9.31 ± .404	(12) 9.84 ± .239	(12) 9.32 ± .255	(12) 9.02 ± .240

[a](No. of birds) mean ± standard error.

[b]Mean having different subscripts are significantly different; m, n ($P < 0.05$); x, y ($P < 0.01$).

Table VII. *The Effect of Feeding Rations Containing Aroclors 1221, 1254, and 1260 on Mean Corpuscular Volume, and Mean Corpuscular Hemoglobin Concentration of SCWL Cockerels*

Parameter	Aroclor	Dietary levels (ppm)			
		0	50	100	150
Mean corpuscular volume (μ^3)	1221	(11) 131.4 ± 1.97[a]	(11) 133.9 ± 3.42	(11) 131.9 ± 4.72	(12) 134.8 ± 5.21
	1254	(12) 137.8 ± 4.44	(8) 134.1 ± 5.16	(7) 127.7 ± 3.28	(6) 130.8 ± 3.78
	1260	(11) 131.9 ± 4.72	(12) 134.8 ± 5.21	(11) 138.6 ± 3.09	(11) 134.7 ± 2.43
Mean corpuscular hemoglobin conc. (gm/100 ml)	1221	(11) 33.4 ± 0.30	(11) 32.8 ± 0.46	(10) 33.5 ± 0.33	(11) 33.9 ± 0.43
	1254	(12) 34.0 ± 0.39	(9) 33.9 ± 0.40	(8) 33.5 ± 0.96	(7) 33.6 ± 1.10
	1260	(12) 33.7 ± 0.63	(12) 34.3 ± 0.44	(12) 34.2 ± 0.47	(12) 33.9 ± 0.27

[a](No. of birds) mean ± standard error.

Table VIII. *Percentage of Chickens Exhibiting Hydropericardium[a] when Fed Various PCB's from One Day of Age*

Aroclor	Treatment period (weeks)[b]	Dietary level (ppm)					
		0	25	50	100	150	χ^2
1221	9	0% (11)[c]	–	0% (11)	0% (10)	0% (11)	NS
1242	8 (trial 1)	0% (12)	0% (12)	20% (10)	62% (8)	–	$P < .001$
1242	8 (trial 2)	0% (10)	–	25% (12)	80% (10)	–	$P < .001$
1254	9	0% (12)	–	8% (11)	50% (8)	57% (7)	$P < .05$
1260	9	0% (12)	–	0% (12)	9% (12)	8% (12)	NS

[a] Hydropericardium taken as > 1.0 ml pericardial fluid.
[b] Average at sacrifice.
[c] (No. of birds).

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