PCB LEVELS IN EGG YOLKS ASSOCIATED WITH EMBRYONIC MORTALITY AND DEFORMITY OF HATCHED CHICKS

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The effect of exposing White Leghorn hens for six weeks to 50 ppm of Aroclor 1254 in water was investigated. While egg production decreased temporarily, no significant effects were noted on egg weights or fertility. Incubation of eggs from exposed hens indicated that this polychlorinated biphenyl (PCB) did not interfere with gametogenesis and fertilization. As Aroclor 1254 accumulated in the yolk, however, embryonic development was arrested at progressively earlier developmental stages. When PCB was removed from the water there was an initial rapid decline in the PCB content of the yolk and a slow return of the normal developmental pattern of the embryo. Leg, toe, and neck deformities were present in many of the chicks hatched from eggs in which yolk PCB level was 10-15 ppm or more. The gross appearance of the liver and the presence of hemorrhages suggest that the liver and the vascular elements may be targets for PCB action.

Introduction

The widespread distribution of polychlorinated biphenyls (PCB) in the environment has become a matter of growing concern (Peakall and Lincer 1970, Risebrough *et al.* 1968). This concern is based, not only on the apparent involvement of PCB in the die-off of some fish and waterfowl, the abnormalities noted in young terns from Long Island Sound (Hays and Risebrough 1972), and the reproductive failure of other avian wildlife species, but also on its cumulative character as one proceeds up the food chain to man. Sufficient evidence exists to indict PCB as embryotoxic in rabbits (Grant *et al.* 1971) and to implicate it in chromosomal alterations (Peakall *et al.* 1972), metabolic derangements (Bailey and Bunyan 1972, Platonow and Funnell 1972, Pardini 1971, Vos *et al.* 1971), and dermatological disorders (Vos and Beems 1971) of experimental animals. Yusho, a poisoning caused by PCB contamination of rice oil, has established its toxicity to humans (Kuratsune *et al.* 1971). For an extensive review of PCB toxicity, see Fishbein (1972).

Studies of the acute and sublethal toxicity levels of PCB have been done on a number of avian species and have established the rather high dosage required to kill birds (Koeman *et al.* 1969, McCune *et al.* 1962, and McLaughlin *et al.* 1963). Peakall (1971) has shown

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that egg shell thinning did not occur in ringdoves. Previously, Peakall and Lincer (1970) cited Tucker's unpublished data that following PCB administration to Japanese quail and mallards, not only egg shell thinning but also a temporary cessation of egg laying occurred. A depressed growth rate of newly hatched chicks was noted with PCB levels of 50-150 ppm in feed (Rehfeld *et al.* 1971). The PCB content in the eggs of pelicans and cormorants has been reported (Anderson *et al.* 1969); in the latter case, there is some evidence linking the increase to the birds' feeding habits (Zitko and Choi 1972). In pheasants, while fertility and hatchability of eggs, mortality of adults, and eggshell thickness were apparently not affected by PCB, the total reproductive success was depressed (Dahlgren and Linder 1971). Peakall *et al.* (1972) have found that exposure to 10 ppm of Aroclor 1254 for three months had no effect on the development of eggs of ringdoves; however, six months later embryonic mortality was nearly 100 percent. Scott *et al.* (1971) found a significant decrease in the hatchability of chicken eggs after treatment for eight weeks with 10 ppm of Aroclor 1254; treatment with 20 ppm caused complete failure, with mortality occurring immediately before hatching.

This report presents preliminary data on the effects of six weeks' exposure to 50 ppm of Aroclor 1254 in water upon the egg production of mature White Leghorn hens, the PCB levels in the yolks, the fertility of the eggs, the viability and hatchability of the embryos, and the deformities present in the hatched chicks. Data are also presented for the following 20-week clearance period on PCB content of the yolks and the viability and hatching percentages of the embryos.

Materials and methods

Twenty-four White Leghorn hens, five months old, selected as active layers, were leg-banded and randomly segregated into a control and an experimental flock. Each flock initially had 12 hens plus one rooster. Water and breeder crumbles (egg-laying mash) were supplied *ad lib.* The egg production of each group was between 70 and 75 percent.

Experimental hens were placed on a water ration containing 50 ppm of Aroclor 1254 (Monsanto, St. Louis, Mo. 63121). The water ration was prepared by emulsifying 0.56 g of Aroclor 1254 in 15.0 g of Tween 80 (polyoxyethylene sorbitan mono-oleate: Sigma Chemical Co., St. Louis, Mo. 63118) at 60° C for one-half hour using the Pyro-multi-magnestir (Lab-Line Instruments, Inc., Melrose Park, Ill. 60160). Subsequently, 100 ml of glass-distilled water was added and the mixing continued for another one-half hour. The resultant emulsion was then stirred into 11.5 liters of water prior to being placed in the water dispenser. The average daily intake of water by both the control and experimental hens was recorded over the six-week exposure period and for two weeks thereafter. Egg production was recorded daily, and the eggs were weighed. During the first four weeks, four eggs were randomly selected from those collected daily from each flock, hard-boiled, and stored in a freezer for PCB analysis. After the fourth week this was done weekly.

Eggs not reserved for PCB analysis were placed in an incubator-hatcher and candled on the fifth, twelfth, and seventeenth day of incubation. Nonfertile eggs were discarded. When candling showed the presence of a dead embryo, the egg was opened. From the degree of embryonic development plus the knowledge of the day the egg was set in the incubator, a reasonable estimate of the age of the dead embryo was possible. Fertility, viability, and hatching percentages were determined for each week's batch of experimental eggs. Equivalent data on control eggs were obtained during the first four weeks of the study (135 control eggs) and weeks 15-18 of the clearance period (158 control eggs). Percentages of nonviable embryos and hatched chicks were based on the number of fertile eggs in each week's set.

While no experimental hens were sacrificed for analysis of their tissues, spot checks of the blood of four hens on days 11 and 24 on PCB confirmed that it was being picked up. This was no longer considered necessary when yolks continued to show increasing PCB levels. After six weeks, when the hens were returned to the control diet, the rate and the degree of PCB clearance were determined by analysis of the yolk. Viability and hatchability percentages were recorded to determine the relationship between PCB levels of the yolk and its interference with normal development. One experimental hen died during week eight off PCB and a second during week 13 off PCB. Both were autopsied, and their brains, livers, and muscle tissues were analyzed for PCB residues.

To compare the PCB levels in the yolks with those in the hatched chicks, analyses of the liver, brain, and breast muscle were projected. However, in week three on PCB, hatching in the experimental flock ceased and did not resume until several weeks off PCB. Data on PCB levels in hatched chicks will be presented in a subsequent paper.

PCB was extracted from yolks and tissues by the method of Richardson *et al.* (1971). Yolks were used instead of whole eggs because of the lipophilic nature of PCB. Assay was by thin-layer chromatography. G1500 Silica Gel acid-fast plates (Shleicher and Schuell, Inc., Keene, N.H. 03431) were spotted with samples and standards and were developed in Nanograde hexane (Mallinckrodt). Subsequently plates were sprayed with silver nitrate dissolved in water and glycerol and then irradiated with ultraviolet for 15 min. Spot densities were determined on a VIS-UV Chromatogram Analyzer (Farrand Optical Co., Inc., Mt. Vernon, N.Y. 11151) using the wide slit. Details of the modified method for PCB analysis of egg yolks and tissues have been published elsewhere (Bush and Lo 1972).

Results

1. **Weight of hens.** At the start of the study, the average weight of the hens of control and experimental flocks was 3.6 and 3.7 lb respectively. After six weeks' exposure there was virtually no change. At the termination of the study, after some fluctuations, controls averaged 3.5 lb and experimental hens, 3.4 lb. Slight weight loss had occurred in six of the ten surviving control hens and in nine of the ten surviving experimental hens.

2. **Water consumption.** Average daily water consumption per hen over the first two months of the experiment was 212 ml for the control group and 177 ml for the experimental hens. While this difference was present from the start of the experiment, it did not affect egg weights. (Average egg weights during this period were 52.8 g for the controls and 53.5 g for the experimental flock).

3. **PCB levels in dead experimental hens.** The two experimental hens that died during the course of this study had been off PCB for more than seven and twelve weeks respectively. Their livers, brains, and muscle tissues were analyzed for PCB residues, and the levels obtained showed a decline, less in the liver than elsewhere. The levels were (in ppm): liver, 56.2, 42.5; brain, 52.7, 20.0; muscle tissue, 12.0, 3.2. It is as yet unknown whether these are toxic levels in the White Leghorn hen. In three hens sacrificed on the conclusion of the study, PCB residues averaged 2.9 ppm in the liver and less than 0.5 ppm in brain and muscle tissue.

4. **Egg production and weight.** The average daily production of the control flock was 8.6 eggs during the first six weeks and 7.3 eggs in the subsequent twenty weeks. This decline reflected three cases of bumblefoot, as well as the normal decline in egg production occurring during warm weather.

The average number of eggs collected daily from the experimental flock for each week of the study is shown in Table I. Two experimental hens died, the first during week seven off PCB, the second during week 13 off PCB. While no definitive cause could be assigned for these deaths, autopsy findings indicated that the hens had been unproductive for an undetermined time. The loss of these hens and the influence of warm weather, however, are not sufficient to explain the temporary decline in egg production of the experimental flock during weeks 3 to 12 off PCB.

The average weight of eggs from the control and experimental hens over the 26 weeks was 53.8 and 52.9 g respectively. No apparent thinness or fragility of experimental eggs was observed.

5. **Egg fertility.** The percentage of egg fertility of the experimental flock for each week of the study is given in Table I. The consistently high percentages indicate that the PCB does not interfere with fertilization. Fertility of control eggs during the first four weeks of the study averaged 92.3 percent; during weeks 15 to 18 off PCB, 93.0 percent.

6. **Nonviability of chick embryos by week of incubation.** The percentages of nonviable embryos in incubated eggs from experimental hens are given in Table I. The distribution of nonviable control embryos for each of the three weeks of incubation during the first four weeks of the study was 4.6, 0.5, and 10.1 percent. During weeks 15 to 18 off PCB, these control percentages were 5.2, 0.6, and 8.3 percent.

Table I. *Effect of PCB on egg production, fertility, hatching, and embryonic viability of White Leghorn hens*[a]

Week	Average daily egg production of flock[b]	Fertility percentage	Hatching percentage[c]	Nonviable embryo percentage by incubation		
				1 week	2 week	3 week
During 6 wk on 50 ppm Aroclor 1254						
1	9.9	98.0	82.7	8.6	8.7	0
2	9.3	100.0	34.4	1.3	0	64.3
3	7.7	100.0	0	0	9.1	90.9
4	9.0	95.0	0	11.2	40.0	48.8
5	8.4	95.0	0	43.2	56.8	0
6	8.3	95.0	0	98.2	1.8	0
During 20 wk following the exposure period						
1	8.3	85.7	0	100.0	0	0
2	8.4	95.0	0	94.5	5.5	0
3	6.4	100.0	0	24.0	76.0	0
4	8.1	100.0	0	7.0	93.0	0
5	7.1	94.0	11.8[d]	29.4	47.1	11.7
6	6.1	100.0	0	33.3	66.6	0
7	4.4[e]	90.0	0	22.0	33.3	44.5
8	4.9	91.6	0	0	54.5	45.5
9	6.3	100.0	6.2	0	50.0	43.8
10	5.2	91.6	9.1	0	72.7	18.1
11	5.6	100.0	26.6	0	20.0	53.3
12	5.9	100.0	46.1	7.7	15.4	30.7
13	6.0[f]	81.2	61.5	7.7	7.7	23.1
14	7.1	94.1	75.0	6.25	6.25	0
15	6.3	85.7	66.6	8.3	8.3	16.6
16	7.3	94.1	77.7	0	0	22.3
17	7.0	90.8	85.0	5.0	0	10.0
18	6.9	100.0	84.6	7.7	7.7	0
19	7.0	94.4	82.5	11.7	5.8	5.3
20	7.0	95.0	89.4	0	0	5.3

[a]Control values are given in the text under the appropriate headings.

[b]Per group of twelve hens initially, reduced by deaths in weeks 7 and 13 off PCB.

[c]Hatching percentages do not include chicks that "pipped" but died before hatching.

[d]Hatching not normal; shells were removed to assist chick.

[e]One experimental hen died before completion of the seventh week off PCB.

[f]Second experimental hen died before completion of the thirteenth week off PCB.

In incubated eggs from experimental hens, complete mortality of first-week embryos occurred during the first week off PCB (Fig. 1). For chick embryos aged 8 to 14 days of incubation, peaks of high mortality were observed in eggs obtained during weeks 4 and 5 on PCB and again during weeks 3 to 12 off PCB. Third-week embryos also exhibited two peaks of high mortality: in eggs obtained from weeks 2 to 4 on PCB and weeks 7 to 16 off PCB. With the removal of Arochlor-1254-contaminated water from the experimental flock, a withdrawal effect was observed in the percentage and time span that nonviable chick embryos aged 8 to 14 and 15 to 21 days were found.

7. **Hatchability**. The hatching percentages of experimental eggs are given in Table I and are correlated with yolk PCB levels in Fig. 2. For control hens, the average hatching percentage was 84.7 during weeks 1 to 4 on PCB and 83.9 during weeks 15 to 18 off PCB.

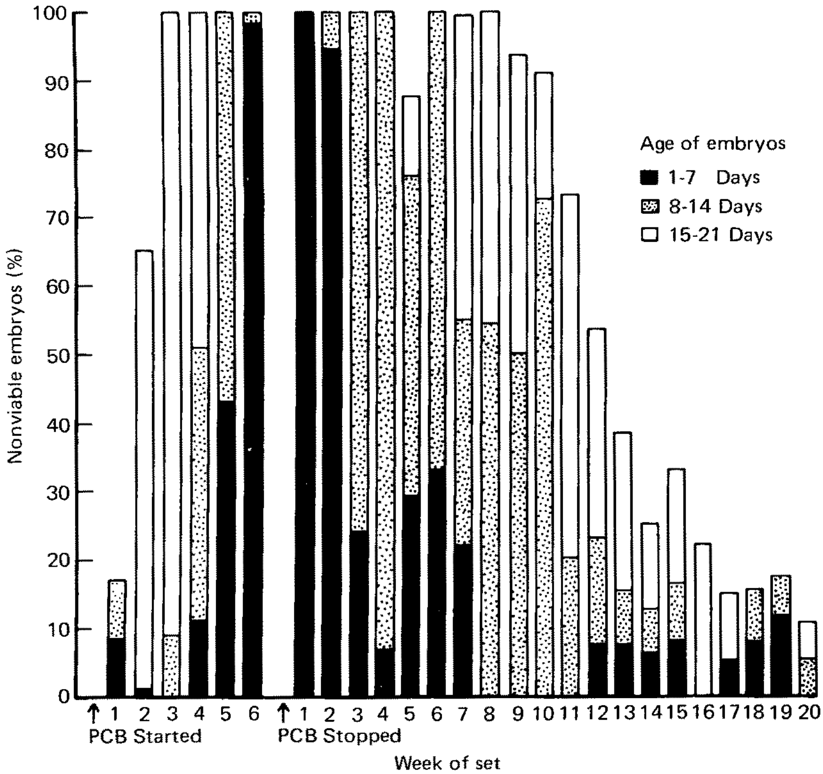

Fig. 1. Total percentage of nonviable embryos for each week's set. Each bar represents the cumulative percentage of dead embryos of incubation ages, one, two, and/or three weeks found in that set.

Hatching of experimental eggs dropped sharply during the second week on PCB. During the next eight weeks, no chicks were hatched. During the fifth week off PCB two chicks were hatched and were assisted by having their shells removed. Four weeks later, normal hatching of chicks resumed, but the number remained low. The percentage increased progressively, however, and by week 17 off PCB it had returned to normal levels.

8. PCB levels in yolks. Fig. 2 shows the PCB levels in yolks of eggs from experimental hens over the 26-week span. During the exposure period these levels increased rapidly. This suggests not only that PCB accumulates within the hen but also that the egg can serve as an excretory mechanism for PCB (Cecil *et al.* 1972, Dahlgren *et al.* 1971). These levels also show a close correlation with embryo nonviability. Moreover, as the PCB level dropped, mortality of the nonviable embryos occurred at a later time during the incubation period (Fig. 1).

It is apparent that PCB clearance is a protracted process (see Fig. 2). When Aroclor 1254 was removed from the water ration of the experimental hens, the yolk levels of PCB

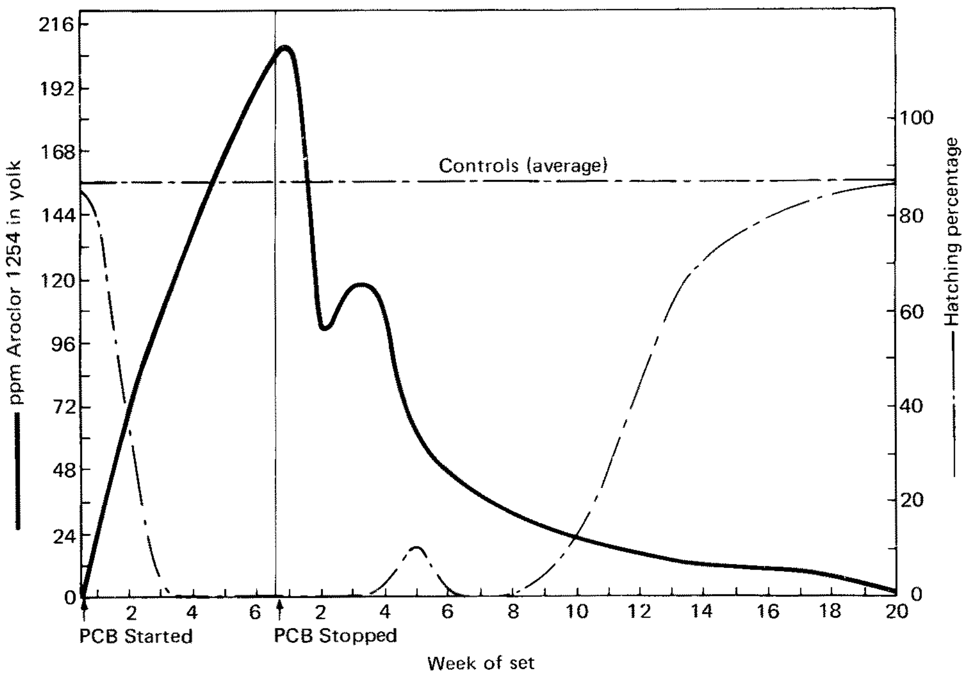

Fig. 2. Relationship between the hatchability of embryos of each week's set of eggs from experimental hens and the average Aroclor 1254 level in yolks of that set.

dropped quickly for five weeks and then more gradually over the remainder of the experimental period. After 20 weeks off PCB, residues in yolk were still present, but only at 0.7 ppm. Since normal hatching percentages were obtained in sets beginning 17 weeks off PCB, it seems apparent that yolk levels under 10-15 ppm have no adverse influence upon chick embryo development.

9. **Condition of chicks hatched from eggs of experimental hens.** Chicks hatched from eggs laid by the experimental hens during the first week of exposure were normal. In the set laid the end of the second week along with the drop in the hatching percentage, a deformity characterized by short, bowed legs and crooked and/or clenched toes appeared in two chicks. During the next 12 weeks (three to six on PCB, one to eight off PCB) only two chicks were hatched, both from the set laid in the fifth week off PCB. Both chicks showed this deformity. Chicks hatching from eggs laid during weeks 9 to 13 off PCB began to show a moderation in the degree of leg and toe deformity and in the percentage of chicks affected. In sets from weeks 14 to 18, toe deformities were usually found only on one foot.

Abnormal curvature of the neck and head position were present in several chicks hatching from eggs laid during weeks 11 to 16 off PCB (Fig. 3). In such chicks from sets

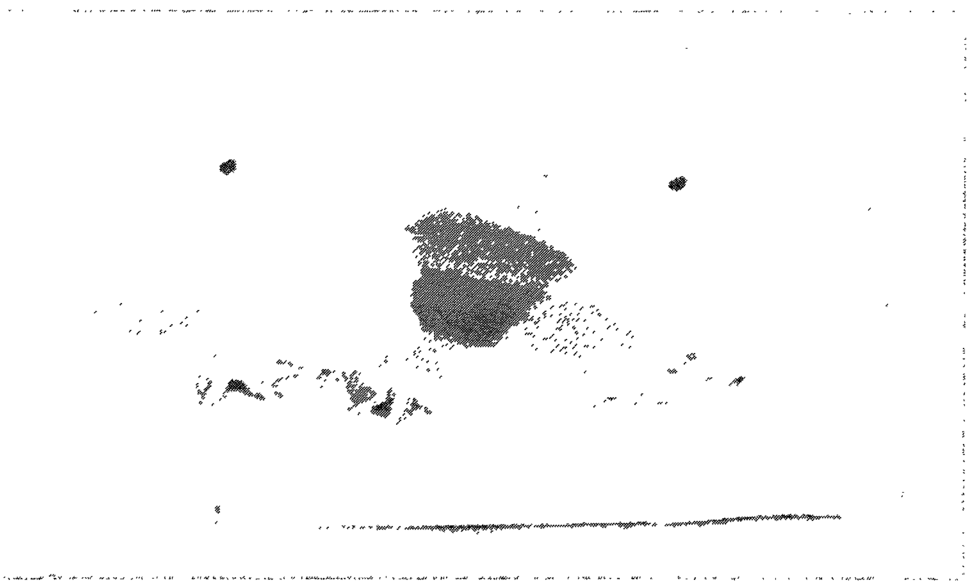

Fig. 3. Toe and neck deformities present in one-day-old chicks hatched from eggs laid by White Leghorn hens 16 weeks off PCB. The incurving of the toes is typical of many of the chicks hatched from eggs of experimental hens during the course of the study.

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In the paper "PCB Levels in Egg Yolks Associated with Embryonic Mortality and Deformity of Hatched Chicks" by C. F. Tumasonis, B. Bush, and F. D. Baker, the photograph on page 319 should be the one shown here.

Fig. 3. Toe and neck deformities present in one-day-old chicks hatched from eggs laid by White Leghorn hens 16 weeks off PCB. The incurving of the toes is typical of many of the chicks hatched from eggs of experimental hens during the course of the study.

laid during weeks 11 and 12, this condition interfered with eating and drinking activity, and the chicks died within a week. In chicks from sets laid during weeks 13 to 16, the condition lasted only two to three days before a near-normal head and neck position was attained. These chicks have survived. This condition was not present in chicks from later sets, although several with deformed toes on one foot were noted. Control chicks showed no anatomical defects.

Other external conditions noted in chicks hatched from eggs laid during weeks 5 and 9 to 12 off PCB were small size; limited walking activity with absence of flight or scatter reaction; difficulty in breathing, deglutition, and eating; irregular, tufty, and dark down feathers; and body tremors. These were not noted in all chicks and were not evident in any chick hatched from eggs laid during or after the thirteenth week off PCB.

On autopsy or sacrifice of chicks hatched from eggs laid during weeks 5 to 12 off PCB, the following conditions were noted: extreme hypotrophy of breast muscle tissue; enlargement of the gall bladder, enlargement and an abnormal brownish yellow color of the liver, small areas of hemorrhage in the muscle tissue, clotted blood in the abdominal cavity, congested cloacal area, and resorbed yolk sac engorged with mucoid material. These anomalies were not always present, and with the exception of the pale color of the liver, they were not found in any chicks from subsequent sets. Many of the conditions noted are in substantial agreement with those found by Rehfeld *et al.* (1972) in one-day-old chicks fed Aroclor 1248.

Discussion

While embryonic deaths do occur during the incubation of normal eggs, the greatly increased percentages of nonviable embryos in eggs from exposed hens during each week of development demonstrate the lethal effect of PCB. The data also show that as PCB levels in the yolk increase, embryonic development terminates at progressively earlier stages. All embryos, however, had developed for at least 48 to 72 hours of incubation, even in sets where mortality of first-week embryos was 100 percent. At this stage, the yolk sac and vitelline circulation have developed sufficiently to begin the transport of deutoplasmic materials to the embryo. The close correlation of the PCB content of the yolks with the nonviability of embryos from these eggs emphasizes the effect of PCB.

One unexplained result of this experiment is the absence of nonviability in the first-week embryos of sets laid during the weeks 8 to 11 off PCB. While the same anomaly in three other isolated weeks (week 3 on PCB, weeks 16 and 20 off PCB) falls within the possibilities of experimental variation, this sustained phenomenon remains baffling.

The manner in which PCB interferes with the developmental process is as yet unknown. Pardini (1971), using beef hearts, has demonstrated in vitro that PCB inhibits the mitochondrial electron transport system on the substrate side of cytochrome C, i.e., NADHcytochrome C reductase and succinate-cytochrome C reductase. Peakall *et al.* (1972) suggests that chromosomal alterations caused by Aroclor 1254 can terminate embryonic development in ringdoves. Some of the toxic actions of PCB noted in chickens (Flick *et al.* 1965, McCune *et al.* 1962, and Vos and Koeman 1970) may also affect tissue and organ anlagen during embryogenesis.

McCune *et al.* (1962) have shown that four weeks' exposure to 100 and 200 ppm PCB caused no mortality in chickens. Flick *et al.* (1965) have reported that 3 out of 24 chicks died during three weeks' exposure to 400 ppm Aroclor 1242. Rehfeld *et al.* (1972) have concluded from their studies of the effects of Aroclor 1248 that the maximum tolerance of one-day-old chicks is approximately 50 ppm. McLaughlin *et al.* (1963) have shown that 25 mg of Aroclor 1242 injected into the yolk sac of chicken eggs resulted in complete mortality; 10 mg resulted in 95 percent failure to develop, and in teratogenic effects. Cecil *et al.* (1972) have studied the effects of a number of PCBs in laying hens. With 2 and 20 ppm of Aroclor 1254 in feed for eight weeks, egg weights and fertility remained unchanged, but hatchability and egg production declined. Reduction in hatchability was greater with 20 ppm of Aroclor 1232, 1242, and 1248 in feed than with 20 ppm of Aroclor 1254. These studies suggest that the toxicity of PCB is age-, dose-, and type-related.

While PCB can be retained and accumulated in the body with chronic exposure (Berlin 1970), their elimination does occur metabolically (Bagley *et al.* 1970, Cecil *et al.* 1972, Koeman *et al.* 1969, Rehfeld *et al.* 1972, Reynolds 1969) as well as by excretion (Cecil *et al.* 1972, Dahlgren *et al.* 1971). Figure 2 and data on PCB levels in autopsied and sacrificed hens suggest that effective PCB clearance can be attained. Although chromatographic studies demonstrate that PCB is metabolizable (Bagley *et al.* 1970, Cecil *et al.* 1972, Koeman *et al.* 1969, Rehfeld *et al.* 1971, and Reynolds, 1969), only minor changes in Aroclor 1254 take place in the cockerel (Platonow and Funnell 1971). Preliminary analytical studies point to sex-related differences in the metabolism of Aroclor 1254 in White Leghorn hens and roosters (Bush and Dell'Acqua 1972).

Our observations on chicks hatched from eggs laid during the weeks off PCB are in substantial agreement with several of those by Rehfeld *et al.* (1971) on one-day-old chicks on an Aroclor 1248 regimen. Respiratory and swallowing difficulties were in evidence. Small discrete areas of hemorrhage were noted in muscle tissue, and clotted blood in the abdominal cavity suggested the involvement of PCB in vessel degeneration. Enlargement and discoloration of the liver indicated an effect of PCB on hepatic function. In addition, we have noted deformities in the legs and toes and an abnormal head position and curvature of the neck.

Exposure of White Leghorn hens and roosters to 50 ppm of Aroclor 1254 in water for six weeks did not affect their ability to produce functional gametes, nor did it repress sexual activity. The failure of the embryos to develop and to hatch, as well as the deformities present in hatched chicks, is attributable to the accumulated levels of PCB in the yolk. However, levels found in the yolks after 17 weeks had no adverse effect on embryonic viability. This evidence raises the possibility that excretion of PCB via the egg is a significant cause of the population decline of several species of waterfowl and avian wildlife.

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