

# Embryotoxic Effects of Three PCB's in the Chicken

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The widespread distribution of polychlorinated biphenyls (PCB's) throughout the ecosystems has been demonstrated. Tissues and eggs of several raptorial and fish-eating birds have been shown to contain up to 1,980 ppm PCB's (RISEBROUGH et al. 1968). It has been suggested that declining populations of several species of raptores may be due to the increased levels of PCB's (ZITKO and CHOI 1972). Reduced hatchability of chicken eggs and increased neonatal mortality of chicks has been reported following the feeding of PCB contaminated commercial poultry rations (PICHIRALLO 1971). Eggs from hens placed on a diet containing 10 ppm PCB (Arochlor 1248) for an eight week period were found to contain 3.06 ppm PCB while levels of 37.1 ppm accumulated in the adipose tissues of the hens. Associated with increasing levels of PCB's was a decrease in hatchability. The majority of the embryos died during the twenty-first day of incubation (SCOTT et al. 1971). It is clear from these observations that development of the avian embryo is altered by PCB's. Therefore, it was decided to evaluate the effect of various PCB's on chick development.

## Methods and Materials

Three isomers of PCB's were purchased from Chem Services, Inc.;<sup>1</sup> Arochlors 1242, 1254, and 1260. Solutions containing 0, .25, 1.25, 2.50 and 5.00 mg/cc were made by dissolving the PCB in acetone, mixing with sesame oil, and evaporating the acetone.

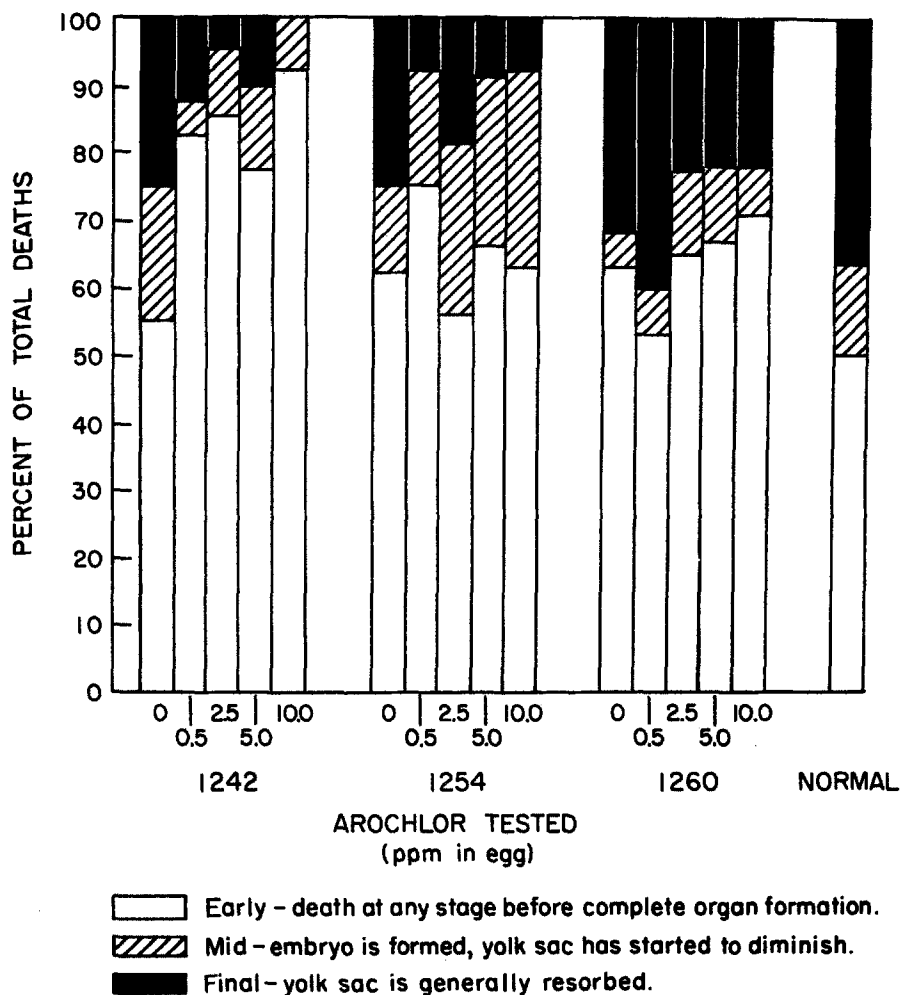
Injections were made by drilling a small hole in the top of the shell with a dental burr, being careful not to pierce the underlying membrane. The shell around the hole was washed with 50% ethanol to insure that a minimum of foreign material was drawn into the egg. One tenth ml. of the desired PCB was injected into each egg with a twenty-two gauge, one inch needle and warm paraffin dropped on the shell to seal the egg. The estimated final concentration of PCB in the eggs was 0, .5, 2.5, 5.0 or 10 ppm. Eggs injected with sesame oil served as controls.

Two experiments were conducted with fertile White-Leghorn eggs weighing an average of fifty grams. The first involved injecting eggs on day zero and incubating them until hatching. Samples of both liver and thyroid were taken of those chicks that hatched while all unhatched eggs were opened and the time of embryonic death noted. The second experiment included groups as positive and negative controls and one group of eggs was injected

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<sup>1</sup>West Chester, Pa. 19380.

Fig.I *Time of embryonic death following injection of PCB's into chicken eggs on day zero of incubation.*



after nine days of incubation. Chicks hatched from these groups were allowed to grow for 14 days and body weights recorded. These chicks were then sacrificed and tissue samples taken for histological examination.

### Results

Hatchability of eggs injected with Arochlor 1242 or 1254 was decreased while the 1260 isomer was without effect (Table 1).

TABLE 1

Effect of PCB's on hatchability when injected  
into the egg on day 1 of incubation

#### Final Concentration of PCB in Egg

Isomer Injected	0	0.5	2.5	5.0	10.0
1242	9/26 <sup>a,b</sup> (23.0)	8/28 (21.4)	6/25 (20.0)	4/30 (11.8)	1/26 (3.8)
1254	12/26 (40.8)	8/20 (40.0)	11/26 (38.5)	8/28 (28.0)	2/25 (4.0)
1260	13/33 (33.0)	8/23 (33.8)	15/37 (31.6)	10/27 (33.3)	13/27 (48.0)

<sup>a</sup>9/26 No. hatched/No. fertile, (expressed as a percent).

<sup>b</sup>Chi-square test for heterogeneity between isomers,  $P < 0.01$ .

The potency of the lower chlorinated isomers is also demonstrated in Figure 1. These results also indicate that the period of organ formation is sensitive to low levels of PCB's as the majority of all deaths occur before organ formation is complete. The results of experiment II (Table 2) support this concept, since injection of the PCB on day 9 of incubation, after organ formation is complete, did not induce embryonic mortality.

The growth of chicks hatched from eggs injected with 5 ppm of Arochlor 1242 on day 0 of incubation failed to grow at a rate comparable to the controls or those treated on day 9. No apparent changes were observed in birds treated with the 1260 isomer on day 0 (Table 3).

### Discussion

The comparative embryotoxic properties of 3 isomers of a polychlorinated biphenyl have been studied. The compounds containing the least amount of chlorine (1242 and 1254) were more

TABLE 2

Embryonic mortality as influenced  
by the time of PCB injection

	<u>Hatchability</u>			<u>Time of Death<sup>d</sup></u>
	<u>No. Fertile</u>	<u>No. Hatched</u>	<u>% Hatched</u>	<u>E--M--F</u>
Normal	35	33	94	1--1--0
Control	38	21	43.5	12--0--5
1242-0 <sup>a</sup>	60	5	8.3	40--9--6
1242-9 <sup>b</sup>	39	32	82.0	2--3--2
1260-0 <sup>c</sup>	32	21	65.5	5--1--5

<sup>a</sup>5.0 ppm PCB injected day zero of incubation.

<sup>b</sup>5.0 ppm PCB injected day nine of incubation.

<sup>c</sup>10.0 ppm PCB injected day zero of incubation.

<sup>d</sup>early-mid-final

early - death at any stage before complete organ formation.

mid - embryo is formed, yolk sac has started to diminish.

final - yolk sac is generally resorbed.

effective in inducing early embryonic mortality than the higher chlorinated compound (1260). The levels affecting hatchability agree with those reported by SCOTT et al. (1971) who fed Arochlor 1248 to hens for varying periods of time and measured PCB levels and hatchability of the eggs. However, they reported that the majority of the embryonic deaths occurred during late stages of development. This is in contrast to our observations that mortality occurred during the period of organ formation. It would appear from our results that PCB's are capable of interfering with inductive mechanisms during the critical period of organ formation. That this is the case is suggested by the data presented in Table 2. Injection of eggs on day 9 of incubation with the same levels of Arochlor 1242 as those injected on day 0 does not affect hatchability. It should also be noted that in embryos failing to hatch, several malformations were noted, such as development of only one eye, hydrocephalus, incomplete abdominal closure and nervous tremors.

TABLE 3

Growth Rates of Chicks Hatched from Eggs Injected with PCB's  
Before or After the Period of Organ Formation

	MEAN <sup>a</sup> TOTAL GAIN			
	Day 3	Day 6	Day 10	Day 14
Normal	5.40±0.64 (10) <sup>b</sup>	16.40±1.12 (10)	63.70±3.91 (10)	78.60±4.83 (10)
Sesame oil	5.20±0.68 (10)	13.40±1.72 (10)	52.60±4.40 (10)	68.30±5.01 (10)
1242-0 <sup>c</sup>	2.20±1.93 (5)	6.60±4.27 (5)	40.75±6.78 (4)	49.25±7.94 (4)
1242-9 <sup>d</sup>	6.10±0.71 (10)	17.20±0.99 (10)	63.30±2.10 (10)	76.50±2.66 (10)
1260-0 <sup>e</sup>	5.80±1.10 (10)	14.20±1.74 (10)	54.55±7.58 (9)	70.00±8.92 (9)

<sup>a</sup>mean weight gain (gms) ± standard error of mean.

<sup>b</sup>( ) number of chicks per group.

<sup>c</sup>5 ppm 1242 injected on day 0 of incubation.

<sup>d</sup>5 ppm 1242 injected on day 9 of incubation.

<sup>e</sup>10 ppm 1260 injected on day 0 of incubation.

Growth rates of birds hatched from eggs injected with 5 ppm Arochlor 1242 on day 0 of incubation were retarded when compared to controls, or eggs treated with 5 ppm 1242 on day 9 or 10.0 ppm 1260 on day 0. Growth retardant effects have been previously reported following feeding of Arochlor 1242 at levels of 200 and 400 ppm during a 3 week period after hatching (FLICK et al. 1965). It is difficult to explain why injections on day 9 of incubation did not influence growth while injections on day 0 severely retarded growth. Differences in liver histology, suggesting a fatty degeneration could account for these observations. It has also been shown that hematopoietic mechanisms are interfered with by the feeding of PCB's (FLICK et al. 1965). It would appear that the growth retardant effects may be due to a combination of effects on the liver and hematopoietic tissues.

The effectiveness of the lower chlorinated compounds has been related to their similarity with the chick edema factor (SCOTT et al. 1971). An alternative suggestion would be that the compounds resemble thyroxine, with chlorine being substituted for the iodine in the 3,5 and 3',5' positions; the PCB could then act as a competitive inhibitor. The observations of SCOTT et al. (1971) that chicks died on day 21 of incubation or shortly after pipping suggests a lack of energy, perhaps induced by a decrease in basal metabolic rate due to a lack of thyroxine. Support for this concept is found in the work of WASSERMANN et al. (1971). They reported that serum PBI levels were significantly reduced in workers occupationally exposed to organochlorine insecticides. However, additional work is required to substantiate our hypothesis.

### Summary

Injection of Arochlor 1242 into chicken eggs on day 0 of incubation severely limited hatchability at levels above 2.5 ppm. The 1254 and 1260 isomers were less effective in this respect, requiring 10 ppm or more. Embryonic mortality occurred during the period of organ formation suggesting an effect on inductive mechanisms. This is supported by observation of several malformations in chicks that developed until day 21. The effects induced by the day 0 injections of the 1242 isomer are permanent as growth rates were severely depressed during the 2 week period following hatching.

### References

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