TOXICITY AND PERSISTENCE OF PCB HOMOLOGS AND ISOMERS IN THE AVIAN SYSTEM

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This study reports the biotransformation and persistence of PCBs in the hen, her eggs, and chicks hatched from these eggs. Twelve White Leghorn hens and one rooster were given 50 μg/ml of the PCB mixture Aroclor 1254 in their drinking water (6.3 mg/kg body weight) for six weeks. The resultant concentration in the yolk of eggs laid during the following 20 weeks and in the tissues of surviving embryos and chicks was determined by thin-layer chromatography and gas chromatography. Mortality of embryos and deformity of chicks were found to be correlated with PCB content of the eggs. Moreover, after storage of the PCB mixture in the hen's body, the toxic potency of the PCB mixture deposited in the eggs more than doubled. It was shown that 3,4,2',3',6'-pentachlorobiphenyl (peak 9) was cleared faster from the hen than 3,4,2',4',5'-pentachlorobiphenyl (peak 12) and 2,3,4,2',4',5'-hexachlorobiphenyl (peak 14) and that 4,4'-chlorination is more important than degree of chlorination in determining persistence of chlorinated biphenyls in the hen, embryo, and chick.

Of the polychlorinated biphenyl (PCB) mixtures manufactured in the United States, Aroclors 1248, 1254, and 1260 (Monsanto Company, St. Louis, Mo. 63166) have the highest acute toxicity for chickens, quail, and rats (Vos and Koeman 1970, Vos *et al.* 1971). Their toxicity to humans has been known since 1937 (Drinker *et al.* 1937). Nevertheless, they have been widely employed as plasticizers, heat transfer fluids, insulators, adhesive additives, and for many other functions, since approximately 1930. The chemical inertness of these compounds has resulted in a wide distribution in the biosphere and in tissues of most forms of life (Biros *et al.* 1970, Fishbein 1972, Hammond *et al.* 1972). Finklea *et al.* (1972) found PCB concentrations up to 29 ng/g in 43 percent of 723 plasma samples collected from healthy volunteers in urban and rural areas of the United States.

Aroclors are complex mixtures of chlorinated biphenyl isomers, the identity, metabolic fate, and toxicity of which are related to the degree of chlorine substitution (Webb and McCall 1972). Biros *et al.* (1970) showed that two samples of human adipose tissue contained substantial quantities of PCBs ranging from penta- to decachlorobiphenyl and including at least 14 isomers and chlorine homologs. Chromatographic resolution of Aroclor 1254 standards, as well as extracts of various cockerel tissue samples, resulted in 13 distinct fractions appearing as well-defined peaks (Platonow and Funnell 1972). Gas chromatograms of Phenoclor DP6 on silicone oil OV-1 revealed 12 peaks with two hexa- and heptachlorobiphenyls as major constituents (Tas and de Vos 1971).

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Sissons and Welti (1971) and Welti and Sissons (1972) have made an exhaustive study of PCBs using the hydrocarbon phase Apiezon L, which gives the largest number of peaks of any stationary phase. Employing support coated open tubular (SCOT) columns and using 220 MHz nuclear magnetic resonance (NMR) and mass spectrometry, combined with Kovat's Retention Indices and chemical synthesis, they have identified 76 individual PCBs from the Aroclor mixtures. Sixty-six of these are present to some extent in Aroclor 1254. Bartle (1972) also identified two PCBs from Aroclor 1254 using 60 MHz NMR and mass spectrometry.

Grant *et al.* (1971) have indicated that the liver is the main site of Aroclor 1254 metabolism and that the components are metabolized at various rates. Curley *et al.* (1971) found no significant difference in storage of PCBs by male and female Sherman rats, but they noted that since the general Aroclor pattern was not observed in fat and urine, metabolism or differential absorption may have occurred.

Several authors have studied the effects of PCB mixtures on liver enzyme functions. Litterst *et al.* (1972) related increased cytochrome P-450 activity and induction of hydroxylation by hepatic microsomal enzymes in rats to increasing PCB chlorine content. Bickers *et al.* (1972) found that the degree of induction of mixed-function oxidases (MFO) in rat livers is dependent, at least in part, on the chlorine content of the biphenyl moiety; and Ecobichon and Johnstone (1973) concur with this for rat liver monoxygenase induction enhancement. Bitman *et al.* (1972) and Bailey and Bunyan (1972) conclude that the biological effects of PCB mixtures are related to their chlorine content.

Platonow *et al.* (1972), studying the effects of Aroclor 1254 on the urinary gonadal steroid levels in the boar, showed that it lowers the levels of dihydroepiandrosterone and estrogen and estrogen metabolite excretion. Their report suggests that PCBs have a deleterious effect upon reproductive activity. Platonow and Funnell (1971) had already indicated the antiandrogen-like effect of PCBs on the cockerel. Koch *et al.* (1972) proposed that ATPase inhibition may be another important effect of PCBs on fish tissue, while Bruckner *et al.* (1973) concurred with Litterst *et al.* (1972) that alterations in the rate of metabolism caused by PCBs may affect the biological responses of mammals to drugs and environmental stress.

The present study was aimed at determining the effect of a low-level intake of Aroclor 1254 on the embryonic development of one species and the biotransformation and persistence of homologs and isomers in the system during and after exposure. The domestic hen was chosen for the study because chicks are particularly sensitive to PCBs (Vos and Koeman 1970) and because the level of PCBs to which the developing embryo is exposed can readily be determined by analysis of the eggs. Experimental data on the effects of this PCB exposure on egg production, fertility, embryonic mortality, and morphological changes have been published (Tumasonis *et al.* 1973).

Experimental design

A low intake of an emulsified PCB mixture in drinking water was planned so that a slow build up would occur in the flock. The chief biological effect measured was em-

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bryonic mortality. When this reached 100%, exposure was terminated. Analysis of eggs indicates approximately the level stored in the hens' adipose tissue. Because of the relative simplicity of the technique, thin-layer chromatography (TLC) was used to determine the total mass of all PCB homologs and isomers present in the eggs and tissues analyzed. Gas chromatography (GC) was used in selected cases, at 1, 11, 16, and 25 weeks for detailed analysis of the variation in the relative proportions of the various homologs and isomers. By analyzing the eggs, embryonic tissues, and chick tissues, the fate of the individual PCBs could be traced from hen to chick during the buildup and decline phases of the experiment, and these data could be related to the observed effects on mortality and chick deformity.

Materials and methods

Dosage, exposure, and handling of the hens. Control and experimental flocks were each made up of 12 White Leghorn hens (age 20 weeks, mean weight 1.65 kg) and one rooster of the same age. The experimental flocks were continuously exposed over a six-week period to 50 parts per million (μg/g) of Aroclor 1254 (6.3 mg/kg of body weight) emulsified with 0.15% Tween 80 in their drinking water. Subsequently they were returned to ordinary tap water and observed for an additional 20 weeks.

Thin-layer chromatography. Analyses of PCB levels in egg yolks and tissues involved the extraction procedure of Richardson *et al.* (1971) and the cleanup and TLC assay methods of Bush and Lo (1973). Selected extracts were subsequently analyzed by GC. Egg whites were not analyzed because they were found to contain <5% of the total PCBs in the whole egg. The eggs were hard boiled before freezing. This facilitated separation of yolk from white and grinding with anhydrous sodium sulphate without affecting PCB contents (Bush and Lo 1973).

Gas chromatography. The Hewlitt Packard (Avondale, Pa. 19311) 7600A Chromatography System was used. The column was 1.5-m × 2-mm i.d. glass, packed with 2% Apiezon L on GasChrom Q (80-100 mesh) and operated at 205°C. The carrier gas was argon and methane (5%) at 40 psi; the flow rate, 60 ml/min.

Eighteen distinct chromatographic zones were measured in each sample using the integrator. To display the resultant mass of data meaningfully, a new graphic presentation was devised. Each peak was compared with the corresponding peak in a standard chromatogram produced by a known mass of Aroclor 1254 to give a value with dimensions μg/g. The 18 values were displayed as equally spaced bars, and an unmodified residue yielded a set of bars of equal height. Because the values of the samples varied widely over the experiment, the bar heights were plotted logarithmically (Figures 4 and 5). Changes in proportions of the 18 peaks are readily discerned. The arithmetic mean of the peaks in each set is represented, on the same scale, by the last bar, giving an estimate of the total mass of PCBs present. This estimate agrees with that given by TLC for unmodified Aroclor 1254 (Bush and Lo 1973). The various methods for quantitation of modified commercial mixtures which have passed through biological systems will be considered in detail in a separate publication (Bush *et al.* 1973).

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Peaks were identified on the integrator output by retention times; tolerance allowed was ±0.10 min. The peaks were measured by area counts (minimum count 500) given by the integrator, entering *via* a Wang data plotter. The nominal μg/g for each peak was calculated as follows:

$$\frac{P_u}{P_s} \times \frac{N}{V_1} \times \frac{V_d}{F} \times \frac{V_2}{V_1} \times \frac{1}{W}$$

where P_u = area of a peak in the sample
P_s = area of the corresponding peak in the standard chromatogram, within five hr of the sample chromatogram
N = weight (ng) which produced the standard chromatogram
V_1 = the volume (μl) injected into the gas chromatograph
W = weight (g) of tissue taken
F = proportion of 100 μl of cleaned-up extract solution remaining after TLC assay
V_d = volume (ml) to which the cleaned-up extract solution was diluted
V_1 = volume (ml) taken for further dilution
V_2 = final volume (ml) after further dilution

The calculation was performed on a Series 700 Advanced Programming Calculator (Wang, Inc., Tewksbury, Mass. 01876). The bar graphs were plotted on the calculator with software developed by Rolf Olsen and Fa-Chun Lo of this Division from Wang swap program No. T.25 6.10.

Statistical treatment of results (regressions). Curves in Figures 1, 2, and 6 were fitted by the method of least squares to either first- or third-order equations and plotted on the Wang calculator. The program was developed from the Wang Statistical Package by Dr. Kenneth M. Aldous of this Division.

Standard error of estimate. The SEE is

$$\left[\sum \left(\frac{d_i y}{y} \right)^2 \Big/ N \right]^{1/2}$$

where N is the total number of results, y is the ordinate given by the regression, and $d_i y$ is the difference between the ordinate of a point and y (Weast and Selby 1967). In Figure 6 the SEE was calculated using a program for linear regressions developed by Rolf Olsen and Kenneth Aldous of this Division. For Figures 1 and 6, $d_i y$ was measured from the curve by hand.

Results

Gross concentration changes. The PCB concentration was determined by TLC in the yolks of at least two of the four eggs which had been hard boiled and frozen each week. The results are shown in Figure 1. The SEE is ± 20% about the least-squares-fitted line and ± 16% about the hand-fitted line. The analytical error is ± 10% for Aroclor 1254

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(Bush and Lo 1973). The curve for hatchability (Tumasonis *et al.* 1973) is also shown. No reduction in egg fertility was observed (Tumasonis *et al.* 1973).

Over the period of the experiment, the PCB concentration in liver, breast muscle, and brain tissue from surviving chicks or 18-day-old embryos (or both) was measured by TLC. Figure 2 shows the regression in level during the decline phase of the experiment. The line slopes on a logarithmic ordinate are: egg, −0.07; liver, −0.11; muscle, −0.11; and brain, −0.16; with standard errors about the estimated line of ±0.25, ±0.25, ±0.33, and ±0.46, respectively, and coefficients of correlation of −0.8, −0.9, −0.8, and −0.8, respectively.

Gas chromatography. Apiezon L was chosen for this work because Sissons and Welti (1971) have shown that it yields the largest number of chromatographic peaks from commercial PCB mixtures. Moreover, they published a chromatogram obtained with an Apiezon L-packed column, although the majority of their work employed SCOT columns. This packed column chromatogram has allowed us to identify the zones in our chromatogram of Aroclor 1254 (Figure 3). Retention times were calculated for each chromatogram relative to the greatest peak (Table I, peak 9). The structures given in the last column of Table I are those assigned by Sissons and Welti (1971) and Welti and Sissons (1972) to their peaks on a SCOT column. Assignment of these structures to the peaks in our chromatogram is made on the basis of the close correspondence of relative retention times shown in Table I and also by considering the relative proportions of the peaks (Figure 3, Table II), which appear to be identical to those of Sissons and Welti (1971).

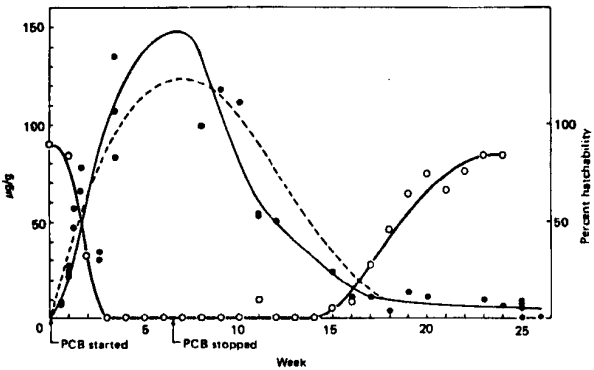

Fig. 1. PCB levels in egg yolk, determined by TLC, and hatchability of experimental eggs. ● = PCB level; ———— = hand-interpolated curve; – – – – = least-squares fit to a third-order regression; ○ = hatchability.

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Figures 4 and 5 show bar graphs selected to illustrate control tissue patterns, analytical recovery patterns, and tissue patterns early and late in the experiment. In control samples of egg yolk and tissues, all peaks were <0.5 nominal μg/g, and all estimates of the level given by the mean of 18 peaks were <0.01 μg/g.

Elimination of individual PCBs. Gas chromatographic data (Figure 3 and Table II) show clearly that the various homologs and isomers are present in the original Aroclor 1254 mixture in widely different proportions. Moreover, peaks 13 and 15 are each a mixture of two isomers, and some of the minor peaks (e.g., 3, 5, and 11) are not well resolved. There is inevitably a large variation between samples because eggs and embryo and chick tissues were taken from a population of discrete individuals related only by their derivation from the one rooster and 12 hens. To identify trends in the data, therefore, somewhat novel data-reduction techniques were resorted to. In the first place, the three major unicomponent peaks (9, 12, and 14) were measured from the bar graphs and plotted against time, using the Wang calculator data plotter, fitting the data to a third-order regression to obtain the curve maximum and extrapolating by hand beyond the point of intersection of the third-order curve with the x axis (Figure 6). Similar treatment of the more minor components was obviously impracticable, so the frequency with which each occurred above or below datum lines drawn on the bar graphs (at the mean and

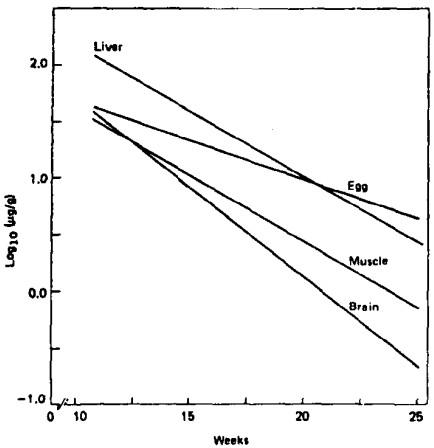

Fig. 2. PCB concentration during the decline phase of the experiment in egg yolk and tissues from 18-day-old embryos and 5-day-old chicks.

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half-mean) was recorded. Table III gives these frequencies, with the total number of analyses concerned, for the major peaks in egg yolk and embryo and in chick tissues. Table IV indicates the persistence of all 18 peaks at four points representative of the whole experimental system.

Discussion

Gross concentration changes. The PCB level in the flock, as indicated by the level in the eggs (Figure 1), builds up faster than it declines. The rate of decline in eggs was exponential, with a constant of $-.07$ weeks^{-1} (Figure 2). When the hatchability curve is compared with the concentration in the eggs (Figure 1), it becomes apparent that the PCB level causing a particular embryonic mortality is lower in the decline phase than in the buildup phase. For example, 50% mortality is caused by 50 μg/g at 1.6 weeks and by only 10 μg/g at 18.7 weeks. Thus the toxic potency of PCBs incorporated into eggs, as

Table I. *Peak Retention Times Relative to Peak 9*

Peak no.	Sissons and Welti (1971) Packed column	Sissons and Welti (1971) SCOT column	Our work	Structures for SCOT column[a]
1	0.40	0.38	0.43	2,5,2′,5′
2	0.44	0.43	0.45	2,3,2′,5′
3	0.51	0.50	0.53	2,3,2′,3′
4	0.58	0.57	0.57	2,5,2′,3′,6′
5	0.64	0.64	0.66	2,3,2′,3′,6′
6	0.69	0.69	0.71	2,5,3′,4′
7	0.80	0.81	0.82	2,5,2′,4′,5′
8	0.91	0.92	0.91	2,5,2′,3′,4′
9	1.00	1.00	1.00	3,4,2′,3′,6′
10	1.13	1.15	1.15	2,3,6,2′,4′,5′
11	1.31	1.32	1.32	2,3,4,2′,3′,6′
12	1.40	1.45	1.45	3,4,2′,4′,5′
13	1.60	1.65	1.64	3,4,2′,3′,4′ + 2,4,5,2′,4′,5′
14	1.80	1.92	1.88	2,3,4,2′,4′,5′
15	2.06	2.00	2.14	3,4,2′,3′,4′,6′
		2.10		2,3,5,2′,3′,4′,6′
16	2.92	2.82	3.11	2,4,5,3′,4′,5′
17	3.14	3.18	3.41	3,4,2′,3′,4′,5′
18	3.56	3.56	3.88	2,4,5,2′,3′,4′,5′

[a] Welti and Sissons (1972).

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measured by its effect on hatchability, is five times greater at 18.7 weeks than at 1.6 weeks. This is expressed clearly in Figure 7, and comparisons of PCB toxicity at seven pairs of points on the hatchability curve are given in Table V. The ratios in the last column express the increase in PCB potency at each pair of points in time. It is not possible to relate those ratios back to the potency of the original Aroclor 1254 mixture, but they show clearly that 11 to 13 weeks after termination of the exposure, the residue in the flock is far more toxic than the residue deposited in the eggs during the buildup phase. Furthermore, this relative increase in potency becomes significantly greater until the residue ages beyond 18 weeks; then its toxicity declines.

This phenomenon has important ramifications when the effect of PCB residues found in species of wildfowl and their eggs is related to the viability of the species. Little effect on the adult birds was observed in this experiment, but the decline level of PCB causing 50% embryonic mortality is well below that reported for Arctic terns (Hays and Risebrough 1972). Present observations indicate the difficulty of interpreting levels of PCB in wildlife because residues may be derived from different commercial mixtures that have been aged for different time periods before reaching the target in question.

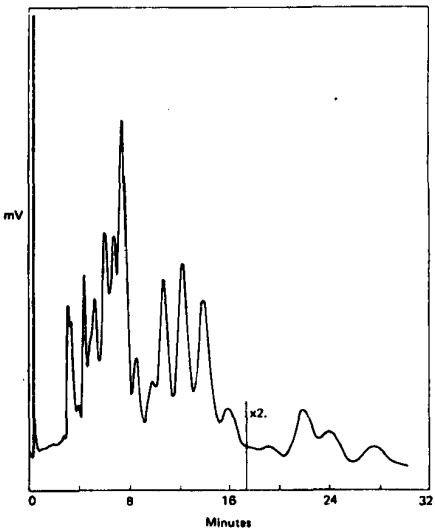

Fig. 3. Gas chromatogram of Aroclor 1254 (75 ng) on Apiezon L.

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There could be two possible causes for the increase in potency of the PCB mixture: an increase in the relative concentration of homologs and isomers which are intrinsically more toxic, or the production of toxic metabolites during the elimination of nonpersistent PCBs. Aromatic molecules such as the PCBs have been shown (Daly *et al.* 1972) to be hydroxylated in the liver to phenols and *trans*-dihydrodiols *via* arene oxide intermediates. Hutzinger *et al.* (1972) have shown that hydroxylated metabolites are produced from pure PCBs by rats and pigeons, but nothing is known of the physiological properties of these PCB derivatives. The possible arene oxide intermediates, which have been shown to be mutagenic in polynuclear aromatic hydrocarbon metabolism (Daly *et al.* 1972), are similarly uncharacterized.

In this experiment deformities of the neck and of the toes were observed in 6 and 33, respectively, of 115 hatched chicks (Tumasonis *et al.* 1973). The neck deformity appears similar to the deformity first noted by Durant (1926) and subsequently shown to be inherited as a simple Mendelian recessive (Knowlton 1929). Crooked toes are also under the influence of genetic factors: however, the genes concerned express themselves differently under different environmental conditions (Black *et al.* 1952). These deformities were not

Table II. *Relative Proportions of Components of Aroclor 1254 Separated by Apiezon L*

Peak	Integrator counts for 100 μg (X 10^3)	% of total
1	8.2	2
2	11.4	3
3	3.5	1
4	16.1	4
5	8.6	2
6	20.1	5
7	32.1	8
8	34.7	8
9	65.9	15
10	20.2	5
11	13.7	3
12	42.9	10
13	55.5	13
14	56.8	13
15	19.2	4
16	4.6	1
17	2.7	0.6
18	1.7	0.4

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present in the pedigree stock from which the birds were taken, in the control flock, nor in 283 chicks hatched from eggs of the control flock.

The deformities may be caused by a teratogenic effect of the PCBs or their metabolites. First generation (F_1) chicks with neck deformities did not survive beyond one week. However, toe deformities have recently been exhibited by 3 of 34 chicks in the second generation (F_2) – that is, chicks hatched from eggs laid by the surviving progeny (F_1) of the hens which had been fed PCB. The mutagenic potential of PCBs suggested by Peakall *et al.* (1972) therefore appears possible. Unfortunately, the present cleanup process

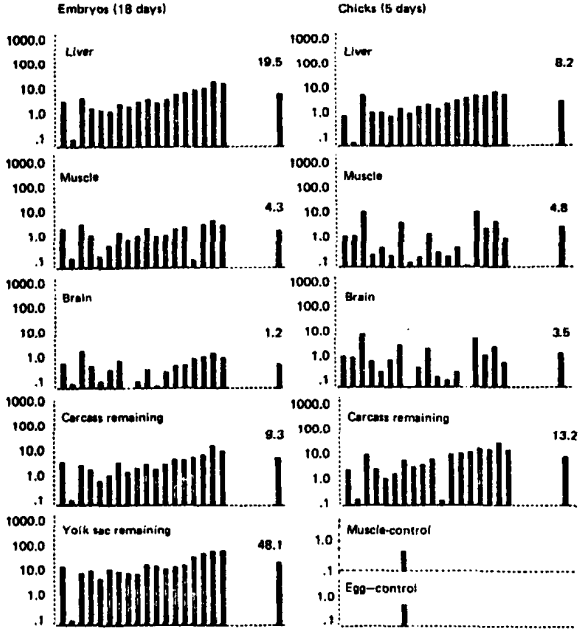

Fig. 4. PCB peak patterns in 18-day-old embryos and 5-day-old chicks from eggs laid at 1 week with pattern of control egg yolk. Logarithmic ordinates are μg/g.

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destroys arene oxides; but the metabolic elimination of particular PCBs can be detected by GC analysis and inferences drawn as to their possible fate.

Gas chromatograms. Spiked control tissue chromatograms (e.g., Figure 5) showed that the cleanup method did not alter the relative proportions of the PCB isomers and homologs in the mixture. Control tissue analyses (Figures 4 and 5) showed an insignificant background in the original flock. One small contamination (<1 nominal μg/g) in muscle and egg coincided with peak 7; one in liver and in brain coincided with peak 8.

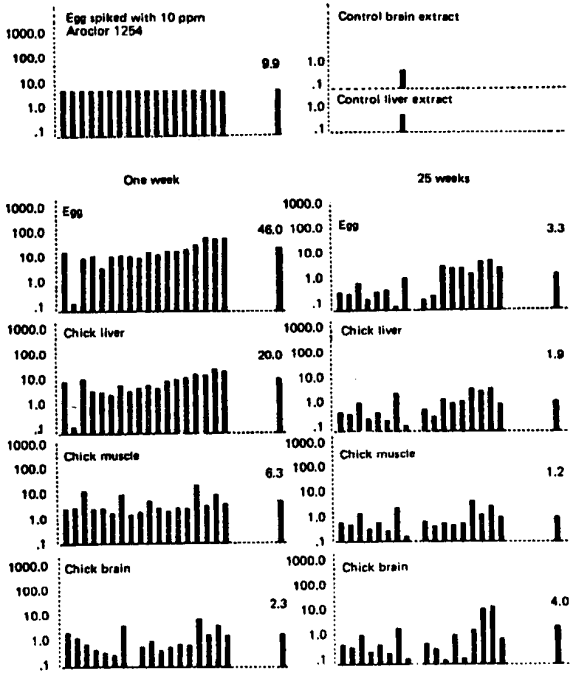

Fig. 5. PCB peak patterns for eggs at one and 25 weeks and chicks derived from them, with patterns for control and spiked tissue. Logarithmic ordinates are μg/g.

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Early in the experiment, the compositions of the PCB mixture in the yolk (Figure 5) and in the residual yolk sac after 18 days of incubation (Figure 4) are remarkably similar, showing that as the embryo absorbs yolk material, it does not absorb preferentially any particular PCB. Moreover, the peak pattern in eggs resembled closely that of the original mixture. However, after 16 to 17 weeks the pattern changed substantially, until the pattern at 25 weeks shown in Figure 5 was reached. The concentration of peak 2 (2,3,2′,5′-tetrachlorobiphenyl) shows the most marked reduction early in the experiment.

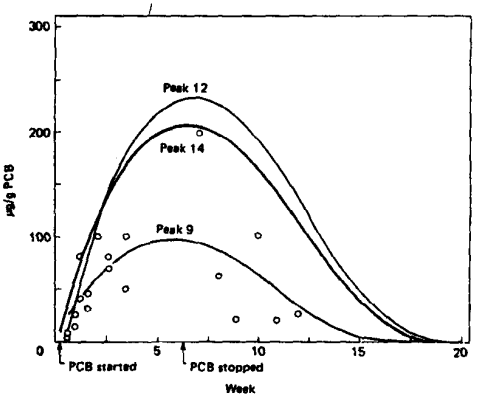

Fig. 6. Nominal PCB concentrations of peaks 9, 12, and 14 in egg yolk during the experiment. For method of calculation, see Results. Standard errors about estimated curves (SEE) are: peaks 9 and 12, ± 0.53; peak 14, ± 0.50. Actual points shown for peak 9 indicate the magnitude of dispersion expressed by the SEE.

Table III. *Frequency of Occurrence (%) of Major Peaks Greater than Half the Mean of Peaks in Each Chromatogram*

Sample	Number of samples	Peaks		
		9	12	14
Egg yolk	2	50	100	100
Embryo	14	21	54	86
Chick	10	10	60	60

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but its appearance in 4 of 13 analyses of egg yolks and tissues derived from the eggs (Figures 4 and 5) illustrates the variability in pattern from sample to sample which appeared throughout the whole experiment.

Of the tissue patterns (liver, muscle, and brain) those of liver most resembled the egg patterns. Each type of tissue appeared to have a characteristic pattern, but with a sample-

Table IV. *Persistence of PCBs in Egg Yolk, Embryo, and Chick Tissues at Weeks 1, 11, 16, and 25, Related to Substitution Pattern and Degree of Chlorination*

Peak no.	Chlorine position	D[a]	Frequency of occurrence > 70%								Total number of vacant positions		
			Less than $\bar{x}/2$ at days				Greater than $\bar{x}$ at days						
			1	11	16	25	1	11	16	25	*para*	*meta*	*ortho*
1	2,5,2′,5′	4	x	x	x	x					2	2	2
2	2,3,2′,5′	4	x	x	x	x					2	2	2
3	2,3,2′,3′	4		x	x				x		2	2	2
4	2,5,2′,3′,6′	5	x	x	x	x					2	2	2
5	2,3,2′,3′,6′	5	x	x	x	x					2	2	1
6	2,5,3′,4′	4	x	x	x	x					1	2	1
7	2,5,2′,4′,5′	5					x		x	x	1	2	3
8	2,5,2′,3′,4′	5	x	x	x	x					1	2	2
9	3,4,2′,3′,6′	5	x	x	x	x					1	2	2
10	2,3,6,2′,4′,5′	6		x	x	x					1	2	1
11	2,3,4,2′,3′,6′	6	x	x	x	x					1	2	1
12	3,4,2′,4′,5′	5			x			x	x	x	0	2	3
13	3,4,2′,3′,4′	5						x	x	x	0	2	3
	+ 2,4,5,2′,4′,5′	6									0	2	2
14	2,3,4,2′,4′,5′	6					x	x	x	x	0	1	2
15	3,4,2′,3′,4′,6′	6					x	x	x	x	1	2	1
	+ 2,3,5,2′,3′,4′,6′	7									0	1	2
16	2,4,5,3′,4′,5′	6					x	x	x	x	0	1	3
17	3,4,2′,3′,4′,5′	6					x	x	x	x	0	1	3
18	2,4,5,2′,3′,4′,5′	7					x	x	x	x	0	1	2

D[a] = Degree of chlorination.
x = Frequency > 70% below $\bar{x}/2$ or above $\bar{x}$, as indicated, where $\bar{x}$ is the mean of all peaks in each chromatogram (Fig. 4 and Fig. 5). All analyses of eggs and of embryo and chick tissues are included. Total number of samples was: week 1, 32; week 11, 8; week 16, 4; week 25, 7.

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to-sample variation too great to allow it to be formalized. Consequently it was not possible to identify the homologs and/or isomers showing most affinity for each tissue type. The relative persistence of the individual PCBs in the whole system, however, could be determined.

Elimination of individual PCB isomers. The regressions of the main unicomponent peaks in egg chromatograms (Figure 6) show clearly that peak 9 (3,4,2',3',6'-pentachlorobiphenyl) is much less persistent than peak 12 (3,4,2',4',5'-pentachlorobiphenyl)

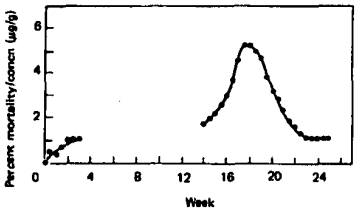

Fig. 7. Variation of toxic potency with time. Percent mortality is mortality at a particular time minus mortality of uncontaminated eggs (8%).

Table V. *Relative Toxicity of PCB Residues in Eggs Early and Late in the Experiment*

Percent mortality	Phase I[a]		Phase II[b]		PCB conc.
	Weeks	PCB conc.[c]	Weeks	PCB conc.[c]	Phase I/Phase II
14	0.5	14	25.5	6	2.3
18	1.0	35	22.8	6	5.8
36	1.5	48	19.4	8	6.0
50	1.6	50	18.7	9	5.6
66	2.0	62	17.4	11	5.6
85	2.5	72	16.3	22	3.3
100	3.0	84	14.0	53	1.6

[a] First 3 weeks of exposure.
[b] Last 12 weeks off PCB.
[c] Values (µg/g) obtained from least-squares-fitted curve, up to 17 weeks; hand-fitted thereafter.

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or peak 14 (2,3,4,2′,4′,5′-hexachlorobiphenyl) in the hen. At the time of greatest PCB potency (17 to 22 weeks), peak 9 had almost disappeared from the eggs; hence, only a metabolite of this PCB could be implicated in the observed enhancement of toxicity to the embryo at this period. Peaks 12, 13, and 14 comprised a large proportion of the residue from 16 weeks to 25 weeks (Figure 5), whereas the potency dropped after 22 weeks, so the possible implication of a metabolite in the toxicity at 17 weeks is increased.

To determine whether embryos and chicks also metabolize the three major peaks, the frequency of their occurrence above the half-mean was recorded (Table III). It is clear that each PCB is being metabolized by the embryo because each peak is found less frequently above the half-mean from week to week. When essentially the same technique was used to determine the persistence of all the peaks in all the gas chromatograms produced in the experiment (to improve the discrimination, two datum lines were drawn at half-mean and mean), a remarkable differentiation between persistent and nonpersistent PCBs was apparent (Table IV).

Furthermore, an analysis of nonchlorinated positions (shown in the last three columns) shows that substitution at the 4- or *para*-position influences the rate at which the PCBs are metabolized. Since the GC separation is achieved by gas-to-liquid partition, the strict correlation of *para*-substitution with GC retention time also shows the importance of *para*-substitution in determining the partition properties of the PCB molecule. Partition phenomena are of extreme importance in the transport of lipids in biological systems.

To discover whether the intrinsic toxicity of a persistent PCB or a metabolite of a nonpersistent PCB is responsible for the enhancement in toxicity of the PCB mixture, future studies should concentrate on pure PCBs with substituted and unsubstituted 4-positions. Difficult syntheses such as 3,4,2′,3′,6′-pentachlorobiphenyl (peak 9) will probably not be necessary. The more readily synthesized symmetrical PCBs will suffice. When the effect of 4-substitution has been ascertained, the search for toxic metabolites such as arene oxides will be facilitated.

Conclusions

The results of this work yield three main conclusions. First, the PCB mixture deposited in the egg by the hen becomes more toxic to the developing embryo after the mixture has been stored by the hen for several weeks. Embryonic mortality of 50% was caused, for example, by a concentration of 50 μg/g of PCB in the egg early in the experiment and by only 10 μg/g 12 weeks after the intake of PCB had ceased. This has considerable significance when attempts are made to correlate the mortality of avian wildlife with PCB residue levels.

Second, of the three major constituents of Aroclor 1254, 3,4,2′,3′,6′-pentachlorobiphenyl (peak 9) is eliminated from the hen more rapidly than 3,4,2′,4′,5′-pentachlorobiphenyl (peak 12) and 2,3,4,2′,4′,5′-hexachlorobiphenyl (peak 14). Hence the observed increase in toxicity to the embryo may be accounted for either by a higher intrinsic

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toxicity of the latter two compounds or by toxic metabolites of any or all three compounds.

Third, chlorination at the 4-position in penta- and hexachlorobiphenyls is associated with slow clearance of PCB isomers from the hen, embryo, and chick. Thus the degree of chlorination may not be the dominant factor in determining persistence.

The results indicate the desirability of further work on pure samples of these three PCBs to determine their intrinsic toxicity and the nature and toxicity of their metabolites. Alternatively, the importance of 4-substitution in the toxicity of PCB could be evaluated by a study of pure PCBs with substituted and vacant 4-positions.

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