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MODIFICATION OF THE HOMOLOG AND ISOMER COMPOSITION OF A POLYCHLORINATED BIPHENYL MIXTURE DURING PASSAGE THROUGH TWO BIOLOGICAL SYSTEMS

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Abstract

The polychlorinated biphenyl (PCB) mixture Aroclor 1254 was administered to breeding populations of hens and rats at 6 mg/kg body weight for 6 weeks. The populations were observed then and during the subsequent 20-week clearance period. Egg yolks and tissues of adults, embryos and fetuses, chicks and pups were analyzed; and the fate of 16 individual homologs and isomers of Aroclor 1254 was observed during the build-up and decline periods. The toxicity of the PCB mixture in the hen can be attributed either to a metabolite of 3,4,2',3',6'-pentachlorobiphenyl or to the intrinsic toxicity of the more persistent 3,4,2',4',5'-pentachlorobiphenyl or 2,3,4,2',4',5'-hexachlorobiphenyl. In the rat, however, both of these pentachlorobiphenyls were eliminated relatively rapidly. Hence 4,4'-substitution is more important in determining persistence in the chicken than in the rat.

Analyses of rat fetuses showed that there was little placental transfer of PCB. However, PCB was passed from mothers to pups as soon as suckling began. By contrast, the chick embryo was exposed to PCB present in egg yolk from its earliest development.

Analyses of geese and duck collected in New York State confirmed results of laboratory studies with regard to PCB persistence. The laboratory results indicate that the concentrations of PCB in eggs of some wild birds, which as reported by other workers are frequently greater than 10 µg/g, may quite possibly be a factor in the reduced reproductive success of these species.

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1. Introduction

The toxicity of commercially produced polychlorinated biphenyl (PCB) mixtures has been fairly well demonstrated and reviewed in recent years (e.g., Fishbein [1], Hammond et al. [2]). The toxicity of the mixtures themselves and of several components of typical mixtures has been determined in a wide variety of living organisms. In long-term low-level experiments, the concentrations of PCB in various tissues of populations of animals have been determined. In short-term experiments, metabolic products of several individual PCBs have been isolated and characterized. In some studies with commercial mixtures, metabolic changes in composition of the ingested mixture have been noted; both changes with time and differences between organs in the same animal have been observed. A more thorough investigation of such changes was one of the primary aims of the present work.

The toxicity of PCB mixtures first drew wide attention in the poultry industry because newly hatched chicks are highly susceptible to the compounds. Since this rediscovery (the toxicity had been clearly demonstrated in 1937 by the U.S. Surgeon General [See 2]), residues of PCB found in wildlife have been blamed for reduction in the reproductive success of various species of birds and of some carnivorous mammals, e.g., otters and mink. Another aim of the present work, therefore, was to investigate the effect of a low-level intake of a common PCB mixture (Aroclor 1254) on the young of an avian and a mammalian species.

2. Experimental

Aroclor 1254, kindly donated by Monsanto Chemicals, St. Louis, Mo., was dissolved in the detergent Tween 80 (Sigma Chemical Co., St. Louis, Mo.) and then emulsified with water. The concentration was adjusted to give the animals a daily intake of 6 mg/kg body weight.

Twelve white Leghorn hens and two roosters were used for the first experiment. Details of the experiment and some conclusions have been reported by Tumasonis et al. [3] and Bush et al. [4] . Wistar rats (125 male, 125 female) were used in the second experiment.

Extraction of PCB from tissue samples and from eggs was carried out by the usual methods (e.g., Bush and Lo [5]). Possible residues of DDT-related insecticides derived from animal feed were eliminated by oxidative clean-up with chromic acid in acetic acid.

Analysis was carried out with 7600A Chromatographic System (Hewlett-Packard, Avondale, Pa.) using electron capture detection. The stationary phase was Apiezon L (2% on Gaschrom Q, 80-100 mesh), and the areas of the eighteen peaks, as identified by Sissons and Welti [6,7], were integrated. For quantitation, the area of each peak was divided by the area of the corresponding peak in the chromatogram produced by a known mass of Aroclor 1254. This ratio permitted a rough quantitation of the peak in µg/g. We have called each such value the notional concentration of the PCB component in the tissue extract. The mean of these notional concentrations gives an estimate of the total mass of PCB present in the tissue. The notional concentrations and their means were displayed as logarithmic bar charts using the Wang Programmable Calculator and Data Plotter (Wang Inc., Tewksbury, Mass.). An unmodified residue would give a set of 19 equal

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bars (18 peaks plus the mean). The value of each mean is printed above it.

3. Results and Discussion

3.1 The hen experiment

Embryonic mortality reached 100% in eggs laid by hens which had been exposed to the PCB intake for 3 weeks. Exposure was terminated 6 weeks after the commencement of the experiment. Chicks started to hatch again from eggs laid in the 15th week of the experiment, i.e., 9 weeks after termination of intake.

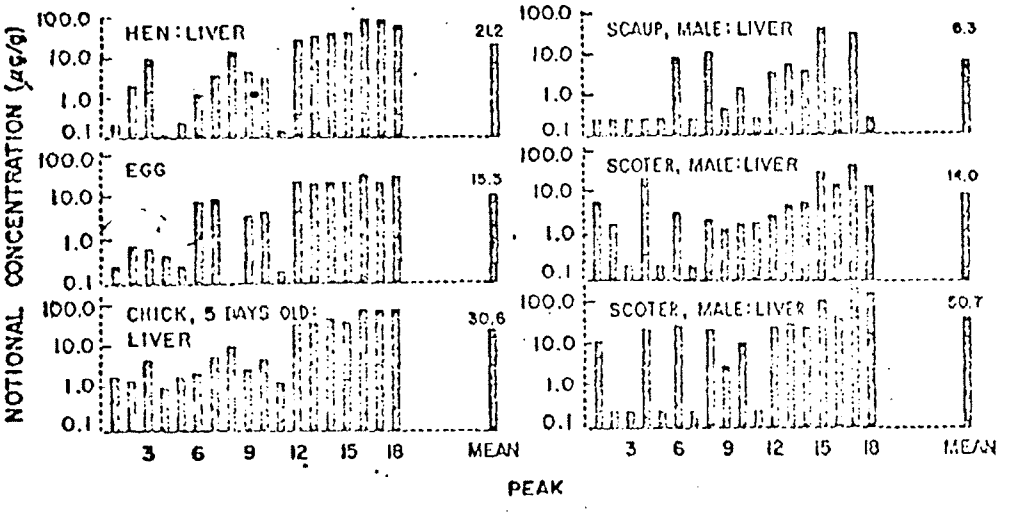

Figure 1. Aroclor 1254 modified by the White Leghorn nine weeks after termination of intake. PCB residues from contaminated New York State waterfowl.

Figure 1 shows the isomer and homolog pattern of residues recovered from a hen, an egg and a surviving chick, all derived from the 16th week of the experiment. It is clear that residence of the PCB mixture in the hens has produced a considerable change in the relative concentrations of the components. Moreover, the PCB deposited in the egg by the hen is incorporated into the chick during its embryonic development. We have earlier shown (Bush et al. [4]) that at this period of the experiment, the residual mixture was up to five times more toxic to embryos than the original mixture and that the increase in toxicity was most probably due to the relatively high concentrations of the components of peaks 12, 13 and 14. The patterns shown in Figure 1 can be used to indicate the potency of residues found in specimens with unknown histories, such as avian wildlife.

We showed previously (Bush et al. [4]) that of the four major constituents of Aroclor 1254, peak 9 (3,4,2',3',6'-pentachlorobiphenyl) did not persist in the hen system, whereas peaks 12, 13 and 14 did persist. After termination of intake, peak 9 soon declined until, at the stage shown in Figure 1, it was reduced ten times relative to peak 12. The ratio of these two peaks might therefore be used as a rough indication of the age of a residue. Figure 2 shows the variation of this ratio (12:9) in eggs laid during the course of the experiment. Despite the large dispersion, a trend is clear. So long as the ratio is below 10, it is probable that such a residue found in an egg or in the liver of a hen or chick is derived from

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an exposure terminated not more than 4 or 5 months earlier (liver composition and egg composition are highly correlated, Bush et al. [4]). Moreover, such a residue would be more toxic to embryos than an older residue containing relatively more of the hexa- and heptachlorobiphenyls.

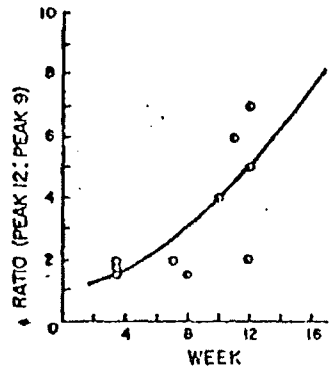

Figure 2. Change in the ratio of the notional concentration of peaks 12:9 in egg yolks with time.

Extrapolation to avian wildlife in general to indicate the absolute potency of a particular residue burden would not be valid. The 12:9 ratio might, however, provide a valuable indication of the age of a residue as one parameter when interpreting the data from surveys of wildlife populations.

3.2 Wildlife survey

In a limited survey of avian wildlife in New York State, two species of duck have been found to be most contaminated by PCB: the greater scaup (Aythya marila nearctica) and the white-winged scoter (Melanitta deglandi). The 17 samples of scaup had a mean level of 5 ± 7 µg/g (mean ± SD) in the liver, with values ranging from zero to 32 µg/g. Eight samples of scoter had a mean breast-muscle level of 7 ± 67 µg/g, with values ranging from 1.5 to 50 µg/g.

Figure 1 shows the residue patterns of three samples of duck liver. All show contamination by PCB mixtures other than Aroclor 1254, as evidenced by the high relative concentration of some of the later peaks and, in the second scoter sample, of several early peaks also. However, there is a distinct resemblance between these patterns and those of hens contaminated with Aroclor 1254. The ratios of peaks 12:9 in the duck samples are 6, 2 and 10 respectively, indicating that the first two probably result from relatively fresh exposure. Thus the mean notional concentration of 14 µg/g in the first scoter sample may be more embryotoxic than the mean of 50 µg/g in the second sample.

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3.3 The rat experiment

The reproductive success of the rats was not affected by the level of PCB intake in this experiment. Intake was terminated after 9 weeks, and the breeding population was observed for a further 16 weeks. Analysis of the tissues of adults, fetuses and pups (both sucklings and weanlings) showed a marked difference in elimination efficiency when compared to the hen. (A detailed report on this study is in preparation.)

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Figure 3 shows typical PCB patterns after 3 weeks of exposure and 4 weeks after termination of exposure, i.e., in week 13. Peak 12 does not persist, and only material eluting after that peak could be implicated in any biological effect on the rat. Two liver microsomal enzymes, aniline hydroxylase and cytochrome P450, were significantly activated by the exposure.

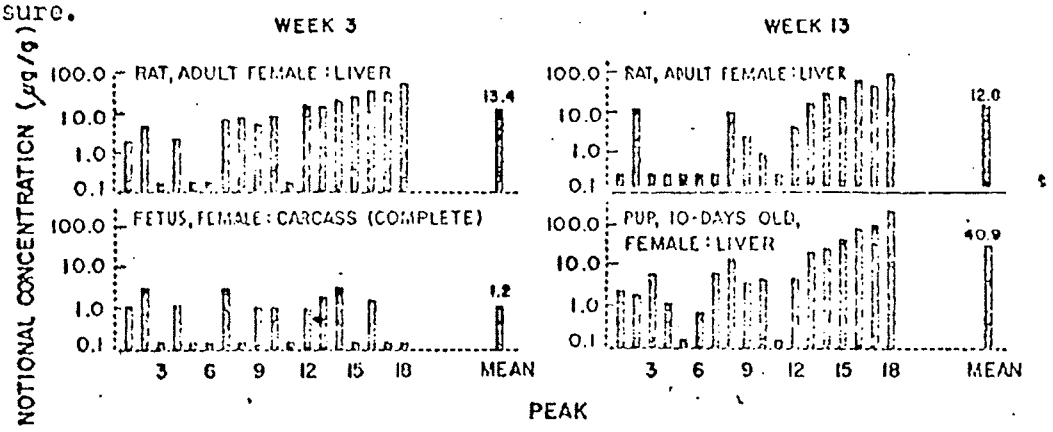

Figure 3. PCB residue patterns in rats after 3 weeks of intake of Aroclor 1254 and 4 weeks after termination of intake.

The major components of Aroclor 1254 are the components of peaks 9, 12, 13 and 14 (Bush et al. [4]) and of these, only peaks 13 and 14 remain in the rat in the 13th week. These represent 3,4,2',3',4'-pentachlorobiphenyl plus 2,4,5,2',4',5'-hexachlorobiphenyl (peak 13) and 2,3,4,2',4',5'-hexachlorobiphenyl (Sissons and Welti [6,7]). It is probable that the first constituent of peak 13 is eliminated because it contains the same substitution pattern on one ring as peak 12; hence the two main components of the residue would have the substitution pattern 2,4,5, and both have 4,4'-substitution. The minimal effect of the PCB intake on the rat and its young may be due to the rat's ability to eliminate all but these two compounds, which may themselves be relatively innocuous.

Analysis of the fetus, however, showed that the lack of effect on fetal viability is probably best explained by protection of the developing fetus from PCB contamination by the placenta. The mean PCB concentration in fetuses was 2 µg/g, in sucklings 50 µg/g and in weanlings 20 µg/g during the experiment. Comparison of these levels with the concentrations in the mothers at the same time (e.g. Figure 4) indicates that little PCB reached the fetus. Only the sucklings became contaminated, and by that stage any possible phase of development which was sensitive to attack by PCB must have passed.

4. Conclusions

The work reported here indicates that there are subtle differences in metabolic efficiency for pentachlorobiphenyls with differing substitution patterns between a mammalian and an avian system. It suggests also the importance of the placenta in protecting the fetus from lipophilic agents such as PCBs. It offers the possibility of evaluating the importance of PCB residues of unknown origin found in wildlife specimens by comparison with model laboratory systems.

The levels reported by other workers in a variety of avian species, if they reflect mixture patterns similar to those reported here, may pose a threat to the early stages of development of the species and hence to their reproductive success.

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