tained an unknown (R_f 0.27) in the alachlor experiment and an unknown (R_f 0.30) in the propachlor experiment (Tables III and IV). According to Lamoureux et al. (1971) and Sharp (1972), these herbicides in the plants were rapidly conjugated with nature products as transitory metabolites. Two of them have been identified as conjugates of glutathion and γ-glutamylcystein. Since model metabolites were not available at the time and these metabolites and degradation products did not accumulate in the food-chain organisms (Tables III and IV), no attempt was made to identify them.

Total ^{14}C was higher in organisms than in water. For example, snail accumulated 24 times more alachlor metabolites than did the water. We do not know whether this constitutes an environmentally acceptable level, but we do know that the accumulation is much less than that of organochlorine pesticides such as DDT which is concentrated over several thousand times more in organisms than in water (Metcalf et al., 1971).

In summary, alachlor and propachlor were rapidly degraded in the water. There was no evidence to indicate that these two herbicides and their degradation products were magnified in the food chain. For example, the total radioactivity in a food chain from algae → mosquito → fish decreased from 0.66 to 0.23 ppm in alachlor, and from 0.21 to 0.015 ppm in propachlor.

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Polybrominated Biphenyls: Tissue Distribution and Effect on Hepatic Microsomal Enzymes in Japanese Quail

John G. Babish, Walter H. Gutenmann, and Gilbert S. Stoewsand*

Japanese quail were fed polybrominated biphenyls (PBB) at 0, 10, 20, 100, or 1000 ppm in a semipurified diet for 9 weeks. They would not accept the diet containing 1000 ppm. Although feed intake and growth were similar in quail fed 0–100 ppm of PBB, egg production was reduced with none of the eggs hatching from quail hens fed 100 ppm of PBB. Tissue residues of PBB were generally higher in males than females. The liver microsomal enzymes, aniline hydroxylase, aminopyrine *N*-demethylase, *N*-methylaniline *N*-demethylase, and *p*-nitroanisole *O*-demethylase were all induced by dietary PBB. Male quail had the highest induction at 20 ppm of PBB, while in female quail peak enzyme induction occurred at 100 ppm of PBB. Post-mortem examinations showed no gross or microscopic lesions in the birds fed up to 100 ppm of PBB.

Polybrominated biphenyls (PBB) have been estimated to have recently contaminated about one million chickens with tissue residues in excess of 1 ppm and egg residues of approximately 0.3 ppm (*Food Chem. News*, 1974). These contaminated flocks have been detected in Michigan as well as other states and are of growing concern to state and federal regulatory and health agencies. The PBB are probably not now present in the ecosystem in as large amounts as polychlorinated biphenyls (PCB) (Peakall and Lincer, 1970) since they have been manufactured as textile flame retardants only since 1970, but their potential toxicity may be greater than PCB (*Food Chem. News*, 1974).

Department of Food Science and Technology, Cornell University, Geneva, New York 14456 (J.G.B., G.S.S.), and the Pesticide Residue Laboratory, Department of Food Science, Cornell University, Ithaca, New York 14853 (W.H.G.).

This investigation was undertaken to study the tissue and egg distribution and excretion of PBB and the effect on reproductive performance and hepatic microsomal enzyme induction in Japanese quail.

EXPERIMENTAL SECTION

Animals and Diet. Japanese quail, 1-day old, obtained from the Department of Poultry Science, Cornell University, were divided into 5 groups, 24 unsexed quail per group within a Petersime brood unit. They were fed ad libitum a semi-purified isolated soybean protein–cornstarch diet (Stoewsand and Robinson, 1970). At 3 weeks of age, calcium and phosphorus were increased in the diet from 1.3 to 3.4% and 0.8 to 1.3%, respectively. PBB (containing about 75% hexabromobiphenyl) was dissolved in corn oil and added to each diet at 0, 10, 20, 100, or 1000 ppm and fed for an additional 9 weeks.

Analytical Procedure. Analytical procedures for the

Table I. Tissue Levels of PBB in Male Japanese Quail

Dietary PBB, ppm	Tissue levels of PPB,[a] ppm				
	Brain	Heart	Kidney	Liver	Muscle
10	26 ± 5.7a[b]	78 ± 46a	51 ± 21a	48a[c]	31 ● 11.7a
20	40 ± 11.2a	59 ± 6.4a	105 ● 23a	374 ± 240b	76 ± 10.9a
100	178 ± 10.3b	627 ± 241b	725 ± 318b	642 ± 360b	235 ± 41b

[a] Values represent means of duplicate analysis ± standard error of moisture-free tissue. PBB recoveries for all tissues range from 95 to 120% with 5 ppm concentration. [b] A common roman letter following the number denotes nonsignificant difference ($P > 0.05$) within tissues. [c] Represents a single pooled sample of six males.

Table II. Tissue Levels of PBB in Female Japanese Quail

Dietary PBB, ppm	Tissue levels of PBB,[a] ppm				
	Brain	Heart	Kidney	Liver	Muscle
10	11 ± 1.2a[b]	48 ± 2.8a	27 ± 10.6a	99 ± 1.6a	15 ± 8.2a
20	26 ± 3.1a	50 ± 21a	43 ± 2.6a	225 ± 59a,b	38 ± 3.4a
100	166 ± 119b	244 ● 74	428 ± 147b	503 ± 119b	351 ± 128b

[a] Values represent means of duplicate analysis ± standard error of moisture-free tissue. [b] A common roman letter following the number denotes nonsignificant difference ($P > 0.05$) within tissues.

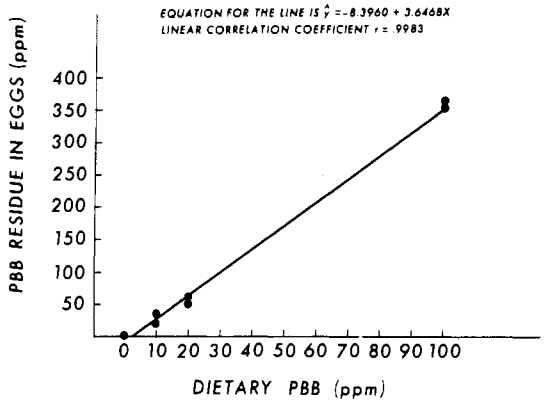

Figure 1. Linear regression analysis of PBB residues in eggs of Japanese quail as a function of dietary PBB. Recovery was 86% at 1 ppm concentration.

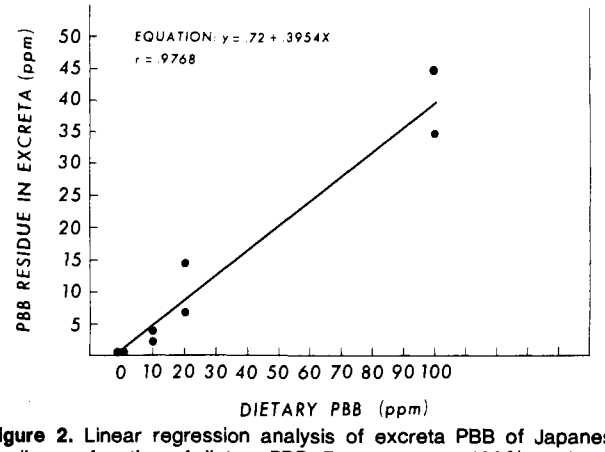

Figure 2. Linear regression analysis of excreta PBB of Japanese quail as a function of dietary PBB. Recovery was 100% at 1 ppm concentration.

tissue, egg, and excreta residues of PBB have been described (Gutenmann and Lisk, 1975).

Microsomal Enzyme Procedure. After killing the animals by cervical dislocation, the livers were quickly excised and weighed. Whole livers (~1 g) were homogenized in 9 vol of ice-cold 40 m*M* Tris-HCl buffer (pH 7.4) using a Potter-Elvehjem Teflon–glass homogenizer fitted to a mechanical drill. The supernatant obtained after centrifuging the 10% homogenate at 9000*g* for 15 min was used for microsomal enzyme analysis and cytochrome P-450 determinations. All enzyme assays were performed on freshly prepared supernatant maintained at 4° throughout preparation.

The 2-ml incubation mixture contained 1 ml of the 9000*g* supernatant (~10 mg of protein/ml), 40 m*M* Tris-HCl buffer (pH 7.4), 0.4 m*M* NADP, 3.0 m*M* glucose 6-phosphate, 2.5 m*M* $MgCl_2$, 7.5 m*M* semicarbazide-HCl when assaying N-demethylation, 4 units of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and substrate in one of the following concentrations: aminopyrine, 7.6 m*M*; *N*-methylaniline, 7.6 m*M*; aniline, 8.0 m*M*; *p*-nitroanisole, 0.2 m*M*. This mixture was incubated under ambient air in 30-ml beakers using a Dubnoff shaking incubator at 37°. All observations were made during a time interval when reaction rates were linear. Reactions were initiated by addition of the substrate and terminated with 1 ml of a 20% trichloroacetic acid solution. For *N*-demethylation studies, aminopyrine and *N*-methylaniline were used as substrates. Activity was determined by measuring the formaldehyde formed (Nash, 1953). Hydroxylation of aniline was determined by measuring the product *p*-aminophenol (Imai et al., 1966). Microsomal *p*-nitroanisole *O*-demethylase activity was determined by measuring the product of the reaction *p*-nitrophenol (Kato and Gillette, 1965). All enzyme activities are expressed as nanomoles of product/milligram of protein per hour.

The quantity of cytochrome P-450 in the 9000*g* supernatant was determined by the method described by Schoene et al. (1972) using a Cary Model 15 spectrophotometer. Under the conditions of the assay, the apparent molar extinction coefficient of the reduced P-450–CO complex is 91 mM^{-1} cm^{-1}.

Protein was determined in duplicate by the modification of the Lowry method (Sutherland et al., 1949). Crystalline bovine serum albumin, fraction V, was used as the standard.

Data were analyzed using analysis of variance and linear regression methods (Snedecor and Cochran, 1967).

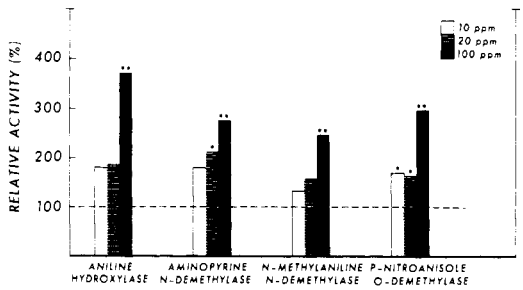

Figure 3. Relative activities of four liver microsomal enzymes in female Japanese quail fed PBB. Controls (0 ppm of PBB) represent the line at 100% activity; significant differences ($*P < 0.05$; $**P < 0.01$).

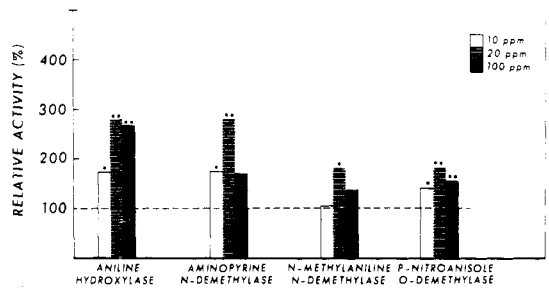

Figure 4. Relative activities of four liver microsomal enzymes in male Japanese quail fed PBB. Controls (0 ppm of PBB) represent the line at 100% activity; significant differences ($*P < 0.05$; $**P < 0.01$).

RESULTS AND DISCUSSION

Japanese quail fed PBB at 1000 ppm refused their diet. Reducing the level of PBB to 500 ppm did not seem to help and all quail within this group died in a few days. Inanition appeared to be the primary cause of mortality.

Tables I and II list PBB tissue residues, per moisture-free tissue, in male and female quail, respectively, after 9 weeks of feeding. The percent recoveries of added PBB to quail tissues ranged from 95 to 120%. With the exception of the liver, in general, the PBB residues were higher in male tissues, reflecting a portion of the PBB residues deposited in the eggs of female quail (Figure 1). About 350 ppm (dry basis) of PBB is present in the eggs of the 100 ppm dietary group. The linear correlation of dietary PBB with egg residues is more than 0.99. Linear correlation of dietary PBB with excreta residues is higher than 0.97 (Figure 2).

It has been established with studies on chlorinated aromatic compounds that the rate of metabolism and excretion is dependent on the amount of chlorine in the molecule. The lower chlorinated isomers are metabolized extremely rapidly (Bailey and Bunyan, 1972; Sosa-Lucero et al., 1973). It remains to be determined if lower brominated isomers would be metabolized and excreted faster than the primarily hexabromobiphenyl compound used in this study.

Unlike chickens fed PCB (Lillie et al., 1974), the Japanese quail fed 10, 20, or 100 ppm of PBB did not exhibit a depressed diet intake or growth retardation. Upon postmortem examination, the quail did not show any gross or microscopic lesions similar to the PBB dosed cow (Gutenmann and Lisk, 1975). Egg production of the quail hens fed 100 ppm of PBB was reduced from about 68% in the controls (0 ppm) to 17%. Lowered levels of dietary PBB (10 or 20 ppm) did not affect egg production, which has also been observed by Cecil et al. (1974) in chickens. No eggs hatched from the quail hens fed the 100 ppm PBB diet; 40% of the embryos died in the first day or two of development.

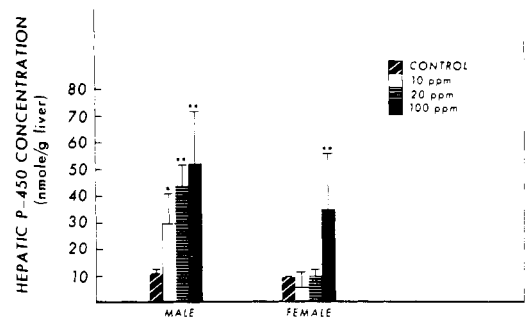

Figure 5. Liver cytochrome P-450 concentrations in male and female Japanese quail fed 0 (control), 10, 20, or 100 ppm of PBB.

Hatchability was not reduced in the 10 or 20 ppm PBB treatment. There was no eggshell thinning in any of the treated groups.

The relative activities of four liver microsomal enzymes in female and male Japanese quail are presented in Figures 3 and 4, respectively. Peak activity of all enzymes was reached at 20 ppm PBB dietary level in the male quail; significant increases ($P < 0.01$) relative to controls for aniline hydroxylase, aminopyrine *N*-demethylase, and *p*-nitroanisole *O*-demethylase activities were observed in the 10 ppm PBB treatment. In the females, peak activities of all liver microsomal enzymes were not reached until the 100 ppm PBB treatment, although induction of *p*-nitroanisole *O*-demethylase occurred at the 10 ppm PBB treatment. The induction of hepatic microsomal enzymes by PCB has been documented in rats (Street et al., 1969; Turner and Green, 1974). Polychlorinated terphenyls (PCT) also induce these liver enzymes, but apparently not until the diet contains 1000 ppm of PCT (Sosa-Lucero et al., 1974).

Similar to the results with PCB-fed rats (Alvares et al., 1973), the microsomal hemoprotein, cytochrome P-450, implicated as the terminal oxidase in the liver enzyme systems, increased linearly in PBB fed male Japanese quail (Figure 5). In the females only the 100 ppm PBB diet induced elevated levels of hepatic cytochrome P-450.

Sex hormones play a role in hepatic microsomal enzyme activity in mammals (Conney, 1967), and the different responses of male and female Japanese quail to PBB intake indicate similar, complex interactions. Male quail liver weights increased linearly ($r = 0.83$) from 1.8 to 2.8% body weight with increased PBB dietary concentration. Female livers, relatively large in controls (4% of body weight), also increased in weight ($r = 0.56$) to 5.3% body weight in the 100 ppm PBB treatment ($P < 0.05$).

It is apparent in this 9-week study that the physiologic response of Japanese quail to PBB is similar in many respects to rat and chicken responses to PCB and PCT. However, quantitative differences occur when the various halogenated aromatic compounds are ingested by mammals or birds.

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Structural Elucidation of the Chlordene Isomer Constituents of Technical Chlordane

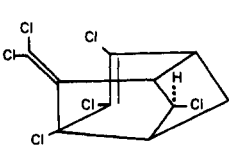
William P. Cochrane,* Harun Parlar, Siegmar Gäb, and Friedhelm Korte

The technical chlordane constituents designated α-, β-, and γ-chlordene are isomers of chlordene ($C_{10}H_6Cl_6$). These isomers are formed via the rearrangement of chlordene by the action of Cl_2 or free-radical initiating agents, and they no longer possess the cyclodiene-type structure. Elucidation of these isomeric structures involved various chemical derivitization reactions. Oxidation with chromic oxide yielded ketones and epoxides while reduction with Zn–HOAc or $CrCl_2$ produced mono- and didechlorinated homologs. Photoisomers, in which the two original ClC=CCl bonds had been cross-linked, resulted from their unsensitized uv irradiation. Spectral confirmation of structure was obtained by ^1H NMR and gas chromatography–mass spectrometry (GC–MS) studies. It is postulated that α-chlordene is 1,2,3,5,7,8-hexachloro-1,3a,4,5,6,6a - hexahydro-1,4-ethenopentalene (III) and β-chlordene is 2,3,3a,4,5,7-hexachloro-3a,6,7,7a-tetrahydro-1,6-methano-1*H*-indene (XIII), with γ-chlordene (XI) being the 2,3,3a,4,5,8-hexachloro isomer of β-chlordene.

Although technical chlordane has been in commercial use for over 35 years, to date only five constituents of this multicomponent pesticidal mixture are known with certainty. These are chlordene, heptachlor, *cis*(α)- and *trans*(γ)-chlordane, and *trans*(δ)-nonachlor (Velsicol Chemical Corp., 1971). Three components, comprising 21–24% technical chlordane, have been designated α-, β-, and γ-chlordene since they are isomers of chlordene ($C_{10}H_6Cl_6$) but their structures have not been elucidated.

The rearrangement of chlordene (Figure 1) in the presence of free-radical initiating catalysts, such as organic peroxides, gives a mixture of α-, β-, and γ-chlordene together with other minor materials, arising from the isomerization reaction (Velsicol Chemical Corp., 1964; Carlson, 1967; Wilks and Richter, 1969). The Velsicol Chemical Corporation introduced this mixture, containing primarily the β and γ isomers, in commercial form (Bandane) in 1967 as an experimental crabgrass herbicide. γ-Chlordene itself can be quantitatively formed by heating the α isomer above 210° for 10 min (Carlson, 1966). Also, the α isomer can add Cl_2 to give a formal isomer of chlordane ($C_{10}H_6Cl_8$), which appears as peak K (referred to as compound K) in the gas chromatogram of technical chlordane shown in Figure 2 (Polen, 1969). In the same chromatogram peak E is a 1:2 mixture of β- and γ-chlordene, while the α isomer occurs between peaks D and E. The melting points and gas chromatographic characteristics, on a mixed OV101/OV210 column of α-, β-, and γ-chlordene, are given in Table I.

Recently, Parlar and Korte (1972) reported on the uv irradiation products of chlordene arising from specific chlorination, dimerization, and ring-opening reactions. Together with photochlordene and heptachlor, two ring-opened products were isolated from chlordene irradiation in acetone. Each of the ring-opened photoisomers of chlordene exhibited two chlorinated double bonds in the 1615–1590 cm^{-1} region of their infrared (ir) spectra ($\alpha_{C=C}$ at 1598 and 1593 cm^{-1}; $\beta_{C=C}$ at 1615 and 1600 cm^{-1}; $\gamma_{C=C}$ at 1614 and 1598 cm^{-1}). Also, unlike chlordene itself, no retro-Diels–Alder fragmentation was observed in the mass spectra. Based on a comparison of the two nuclear magnetic resonance (NMR) spectra it was postulated that the products had structures I and II. However, no verification of these



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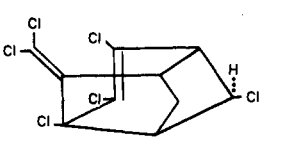

II

structures was reported. The present study was undertaken to determine the structures of the α-, β-, and γ-chlordene components of technical chlordane and compare them with the postulated structures for the ring-opened photoproducts I and II.

EXPERIMENTAL SECTION

The melting point determinations were taken on a Reichert Heizbank (Type 184321, Austria) or a Fisher-Johns hot stage and were uncorrected. A Hewlett-Packard

Analytical Services Section, Plant Products Division, Canada Department of Agriculture, Ottawa, Ontario, K1A OC5 Canada (W.P.C.), and the Institut für Ökologische Chemie der Gesellschaft für Strahlen-und Umweltforschung mbH München, 8051 Attaching, West Germany (H.P., S.G., F.K.).