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Effects of PCBs on Liver Ultrastructure and Monooxygenase Activities in Japanese Quail

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The effect of environmental pollutants such as PCBs and DDT on avian species is well documented (for a review, see Peakall, 1986). It is proven that chronic high-level PCB intoxication perturbs calcium metabolism in birds, affecting eggshell thickness (Call and Harrell, 1974). PCBs have an impact on the liver, which accumulates high levels of toxicants. These induce drug-metabolizing enzyme activities in quail (*Coturnix coturnix*), herring gull (*Larus argentatus*), and partridge (*Perdix perdix*) (Buyan and Page, 1978; Ellenton et al., 1985; Abiola et al., 1989). As these enzymes can degrade endogenous molecules such as steroids, xenobiotics like PCBs can severely hinder birds' reproductive performance. PCBs induce damage such as regression of the testes (Biesmann 1982) decreased sperm concentration (Bird et al., 1983), and altered embryonic development resulting in death or malformation of chicks (Cecil et al., 1974). Moreover, ultrastructural alterations linked with induction of these enzymes have been observed in the livers of PCB-contaminated chickens and ducks (De Los Reyes and Mora, 1978; Williams, 1993).

It is well known that PCB toxicity depends on the structure of the congener considered. The most potent congeners are coplanar and sterically similar to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) (Mc Farland and Clarke, 1989). It is now generally believed that only 15 to 20 congeners are of real concern, but taking only these most toxic congeners into account leads to underestimating the problems associated with the general toxic effects of PCBs in natural ecosystems (Duinker et al., 1991). As most of these highly toxic congeners are present in Aroclor 1254, we have undertaken to study how this mixture affects liver morphology and liver glycogen content in quail. We have further related these morphological modifications to liver monooxygenase activities, so as to obtain valuable *in vivo* reference information on PCB cytotoxicity in quail, to be compared in the future with similar *in vitro* observations on quail hepatocytes.

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MATERIALS AND METHODS

Twenty Japanese quail hatchlings (*Coturnix coturnix japonica*) and five 8-month-old adults were purchased from a local breeding farm (Ferme du Moulin, Liernu, Belgium). After 2 weeks of acclimatization, the hatchlings were divided into 5 groups. Control groups consisted of five individuals sacrificed after 22 days (35-day-old specimens) and five individuals sacrificed after 44 days (57-day-old specimens). The five 8-month-old (240-day-old) specimens were taken as 'old' controls.

To the individuals of the two test groups, Aroclor 1254 dissolved in peanut oil was administered 22 times *per os* so as to obtain a theoretical concentration of 50 mg PCBs per kg live weight. Five of these birds were contaminated every 48 hours over a 44-day period, while the remaining five were contaminated daily over a 22-day period.

Just after sacrifice, the birds were dissected and their livers fixed in glutaraldehyde, postfixed in OsO_4, rinsed, dehydrated, and embedded in epoxy resin as described by Thomé et al. (1995). Thin sections contrasted with uranyl acetate and lead citrate were examined with an electron microscope at 80 kV accelerating voltage.

PCBs in the liver were analyzed and quantified according to Hugla et al. (1995). Sample clean-up and congener identification were performed according to Thomé et al. (1995).

The glycogen content of the liver was measured spectrophotometrically as in Seifter et al. (1950).

P450 microsomal concentrations were dosed according to Omura and Sato (1964). Aldrin epoxydase activities were measured following the method of Wolff et al. (1979).

Values of PCB contamination, enzyme activities, and glycogen content were compared in control and experimental groups by means of the ANOVA and Student-Newman-Keuls tests (SigmaStat® 1.0, Jandel Scientific).

RESULTS AND DISCUSSION

The PCB levels measured in the livers of control and contaminated quail are presented in Table 1. The levels measured in control quails on days 35 and 57 are not significantly different. In the two groups of contaminated birds, hepatic PCB concentrations are significantly higher than in controls; quails intoxicated every two days show slightly higher levels than individuals contaminated daily, but the difference is not significant.

The cytochrome P450 concentrations are identical in the two control groups (Table 1). The livers of contaminated quails show a very significantly increased P450 content, whether the animals were treated daily or every two days. The increase is significantly higher in birds treated daily when compared with birds contaminated every 48 hours.

Table 1. PCB concentrations in the livers of control and PCB-contaminated quails (every 24 or 48 hours) in relation to the cytochrome P450 and glycogen contents and to the aldrin epoxidase activity levels (means ± standard deviations).

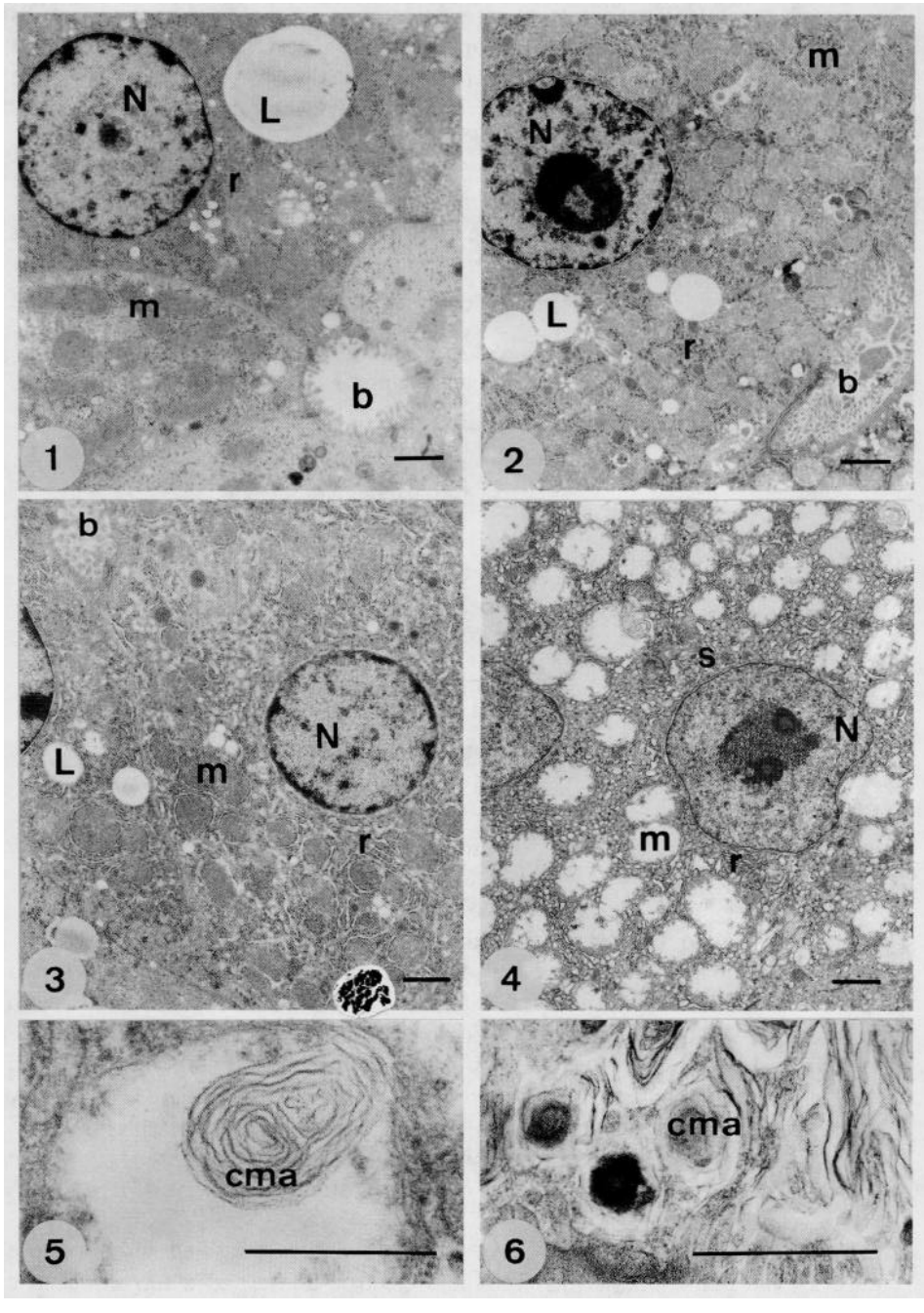
Quail group	Day	PCB concentration (µg/g F.W.)	P450 concentration (pmol/mg prot.)	Aldrin epox. activity (pmol/mg prot . min)	Glycogen content (µg/g F.W.)
Control	35	0.2 ± 0.1	184 ± 58	156 ± 79	261.8 ± 54.1
Control	57	0.2 ± 0.1	139 ± 54	-	-
Control	240	-	-	-	96.5 ± 50.1*
Contam./24 h	35	25.0 ± 5.8*	666 ± 162*•	1528 ± 440*•	74.3 ± 37.2*
Contam./48 h	57	34.3 ± 6.9*	346 ± 85*	922 ± 246*	44.5 ± 18.5*

F.W. : fresh weight; - : no data.
* Statistically different from the corresponding control (ANOVA : $P<0.001$; Student-Newman-Keuls test : $P<0.05$).
• Statistically different from the other contaminated group.

Aldrin epoxidase activity is likewise markedly induced in contaminated quails (Table 1). Induction is again significantly higher in the more frequently contaminated individuals.

The livers of young control quails exhibit a classical organization (Figure 1). Contrary to mammalian livers, however, they exhibit no clearly limited lobules, and the different vessels are irregularly distributed. The bile ducts appear normal, with abundant microvilli; they sometimes contain secretions. The cytoplasm contains a spherical nucleus, with characteristic granular chromatin and nucleoli, and numerous mitochondria with well-shaped cristae. These organelles are encircled by a normal rough endoplasmic reticulum (RER) arranged in parallel layers. Lipid droplets are often stored within the cytoplasm. In control individuals, smooth endoplasmic reticulum (SER) and Golgi complexes are never observed.

The hepatocytes of old control quails exhibit a clearly modified ultrastructure (Figure 2): the mitochondrial matrix is less electron-dense and a few concentric membrane arrays (CMAs, also called myelin-like figures) appear mainly in the bile ducts or within the cytoplasm.



Figures 1-6. Electron micrographs (TEM) of quail hepatocytes.
Figure 1 : control quail (35 d). Figure 2 : control quail (240 d). Figure 3 : quail contaminated with Aroclor 1254 every 48h for 44 days. Figures 4, 5, 6 : quail contaminated with Aroclor 1254 every 24h for 22 days. Bar = 1 µm.
N : nucleus; r : rough endoplasmic reticulum; s : smooth endoplasmic reticulum; b : bile duct; m : mitochondria; L : lipid droplet; cma : concentric membrane array.

Only a few morphological alterations appear in the livers of birds contaminated every other day: a moderate proliferation of SER, the appearance of CMAs in the cytoplasm (Figure 3). More marked degenerative changes in liver ultrastructure are seen in quails intoxicated daily. In response to the exposure, the mitochondrial cristae and matrix regress to total disappearance (Figure 4) and the general shape of some nuclei becomes irregular. The SER and, to a lesser extent, the RER fill the cytoplasm, and their cisternae are markedly dilated. Moreover, there are many CMAs within the empty mitochondria (Figure 5) and in the bile ducts (Figure 6).

Whether PCBs affect the lipid and glycogen contents cannot be deduced from the ultrastructural examination. The glycogen assay, however, shows a higher glycogen content in the livers of young control quails than in those of 8-month-old specimens (Table 1). PCB contaminated birds, furthermore, exhibit a significantly lower glycogen content than the young controls, but there is no significant difference between the contaminated birds and the 'old' controls.

The present study clearly demonstrates the impact of PCBs *in vivo* on liver detoxicating activity and ultrastructure in the Japanese quail. Our enzymatic results are in good agreement with the considerable body of existing data concerning PCB-triggered induction of hepatic enzymes in birds (Riviere et al., 1985; Peakall, 1986). We observe, moreover, that monooxygenase activities respond more markedly to daily than to discontinuous contamination, despite the very similar PCB concentrations measured in the liver.

In young control quails, the organization of the liver is quite similar to that of other birds like chicken (De Los Reyes and Mora, 1978) or duck (Williams et al., 1993). The observed lack of SER, however, does not tally with observations on control ducks (Williams et al., 1993). In 'old' control quails, liver morphology is strongly altered.

Like the monooxygenase levels, the alterations of liver ultrastructure induced by PCBs clearly correlate directly with the frequency of PCB administration, being more severe when the PCBs are administered daily. These levels do not correlate with the concentration of PCBs in the liver, which remains about the same whatever the frequency of PCB administration.

The PCB-induced changes clearly resemble those observed in 'old' control individuals; we can therefore hypothesize that PCBs cause an acceleration of cell metabolism leading to premature cell aging.

SER development following intoxication, as reported here, has previously been observed in PCB-treated ducks (Williams et al., 1993) and chickens (De Los Reyes and Mora, 1978). This phenomenon is a well-known response of organisms to various pollutants (Heinomen et al., 1982) accounting for the induction of hepatic detoxication enzymes (Klaunig et al., 1979).

In quail, the mitochondria1 cristae regress to total disappearance, as observed in pelicans (*Pelecanus erythrorhynchos*) treated with Aroclor 1254 (Stotz and Greichus, 1978). In ducks, however, PCBs seem to have the opposite effect: the mitochondria become more electron-dense as a result of contamination (Williams et al., 1993).

In this work, we have not observed any vacuolization of the cytoplasm of quail hepatocytes, contrary to previous observations on ducks exposed 5 to 50 mg Aroclor 1254 per kg body weight (Williams et al., 1993). As for the development of concentric membrane arrays, this is a classical alteration of the liver also reported for Aroclor-1254-contaminated pelicans (Stotz and Greichus, 1978) mammals, and fish. These arrays are thought to arise from the rolling up of the inner mitochondrial membrane before its elimination by exocytosis into a bile canaliculus (Norback and Allen, 1969). According to Hacking et al. (1977) they may also arise from a reorganization of ER membranes around pollutant-rich lipid droplets providing sites for metabolism and elimination (Chefurka et al., 1987).

In quail, PCBs do not seem to alter the number of lipid droplets; this seems unusual, as reports in the literature mention either an increased lipid content (in PCB-contaminated rats) or a degradation of lipidic material (in PCT-treated rats) (Toftgard et al., 1986).

Apart from this work, deformations of hepatocyte nuclei have been mentioned in only one other *in vivo* study, which focused on sea bass *(Dicentrarchus labrax*) contaminated with benzo(a)pyrene (Lemaire et al., 1992). PCBs have not been found to affect the shape of hepatocyte nuclei in mammals or White Peking ducks (Williams et al., 1993).

The lower glycogen content observed in the livers of PCB-treated quail seems to tally with the decreased number of glycogen patches observed in benzo(a)pyrene- and PCB-contaminated fish (Lemaire et al., 1992; Hugla et al., 1995).

PCBs administered to quail simultaneously increase the detoxicating activity of the liver and alter hepatocyte ultrastructure. These perturbations depend on how frequently the toxicant is administered. They lead to premature aging of the tissue and might also, as demonstrated elsewhere, reduce the reproductive success of the species.

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