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Influence of Cadmium on PCB Congener Accumulation in Quail

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Technological development this century has led to an environmental input of synthetic chemical compounds totally extraneous to natural ecosystems. Polychlorobiphenyls (PCBs), extensively used in agriculture and industry, belong to this class of compounds. These xenobiotics have a high fat/water repartition coefficient, making them easily accumulated by living organisms. Their persistence and low degradability means that they are now present throughout the global ecosystem.

Ubiquitous environmental contaminants also include heavy metals like Hg, As, Pb, Cd and Cr, of which Cd, an element causing functional damage to the kidney and liver in which it preferentially accumulates, is of special ecotoxicological importance. The WHO has defined an accumulation limit of 60 mg/kg (fresh weight), beyond which damage occurs. Cd occurs "naturally" in high concentrations in certain animal species including molluscs. Birds and marine mammals feeding largely on cephalopods show high levels of this metal (Furness & Hutton 1979, Honda *et al.* 1983). Many monitoring studies have also revealed high concentrations of PCBs in the same animals (Renzoni *et al.* 1986, Tanabe *et al.* 1983).

The vast majority of studies on contaminants in experimental animals consider the short-, medium- and long-term effects of a single pollutant. However, the presence of synergisms and antagonisms between compounds makes it necessary to adopt a more holistic approach to the problem of environmental pollution.

In order to understand if the effect of xenobiotic compounds like PCBs may be potentiated by the natural presence of cadmium in bird population, we sought preliminary information on how the presence of cadmium in the diet may influence the accumulation and metabolization of PCB congeners. The influence of Cd on PCB accumulation was investigated in Japanese quail treated experimentally with Cd and PCBs. Particular attention was paid to final congener accumulation and qualitative differences in congener composition between controls and treated birds.

MATERIALS AND METHODS

Twenty-four adult male Japanese quails (*Coturnix coturnix japonica*) of 180g average weight, were kept in single steel cages with commercial pellet feed and water ad libitum and a light period of 12 h/day.

After 20 days, groups of six quails were placed on diets containing 100 μg/g Cd as

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Table 1. IUPAC number and structure of the main PCB congeners.

IUPAC Number	Structure	IUPAC Number	Structure
Pentachlorobiphenyls		Heptachlorobiphenyls	
95	22'35'6	170	22'33'44'5
101	22'455'	171	22'33'44'6
110	233'4'6	180	22'344'55'
118	23'44'5	183	22'344'5'6
		187	22'34'55'6
Hexachlorobiphenyls		Octachlorobiphenyls	
138	22'344'5'		
146	22'34'55'	194	22'33'44'55'
149	22'34'5'6	195	22'33'44'56
151	22'355'6	196	22'33'44'5'6
153	22'44'55'	201	22'33'4'55'6
156	233'44'5	202	22'33'55'66'
		Nonachlorobiphenyls	
		206	22'33'44'55'6

cadmium chloride (Cd group), 100 μg/g PCBs (Arochlor 1260; *see* Tab.1 for congener composition)(PCB group) and 100 μg/g Cd + 100 μg/g PCBs (Cd/PCB group). These diets were suspended after one month and the birds sacrificed. The livers and serum were collected and processed immediately for biochemical analysis. Fractions of liver, kidney and pectoral muscle were kept at -20°C until processing for Cd and PCB analysis. For each group, the Somatic Liver Index (SLI = (liver weight / body weigh) x 100) was determined.

For PCBs, 0.5 g of freeze-dried material was extracted in Soxhlet apparatus with n-hexane; the extract was subjected to sulphuric acid clean-up followed by Florisil chromatography. The analytical method used was high resolution capillary gas chromatography, with electron capture detector (Ni63) and a 30 m SBP-5 bonded phase capillary column. The carrier gas was argon/methane (95:5) at 100 kPa; split ratio was 100:1. Oven temperature was 100°C for the first 10 min, after which it was increased by 3C°/min to 280°C. A mixture of Arochlor 1260, Arochlor 1254 and Arochlor 1016 (1:2:2) (from Supelco Inc., USA) was used for calibration, recovery evaluation and verification. The response factors obtained are consistent with the data in the literature (Mullin *et al.* 1984, Bush *et al.*, 1985; Capel *et al.*, 1985). The results were expressed in mg/kg dry weight.

The greatest extraction of organic substance was obtained from liver (16.4%), followed by muscle (9.0%) and finally faeces (3.7%) in line with lipid content.

The data was processed by summary statistics and ANOVA analysis using Statgraphics software (Statistical Graphics Corporation).

RESULTS AND DISCUSSION

In the control group, PCB concentrations in the muscle were 2,052 ng/g dw (Fig.1). In the PCB group, levels increased to 48,548 ng/g dw. In the PCB/Cd

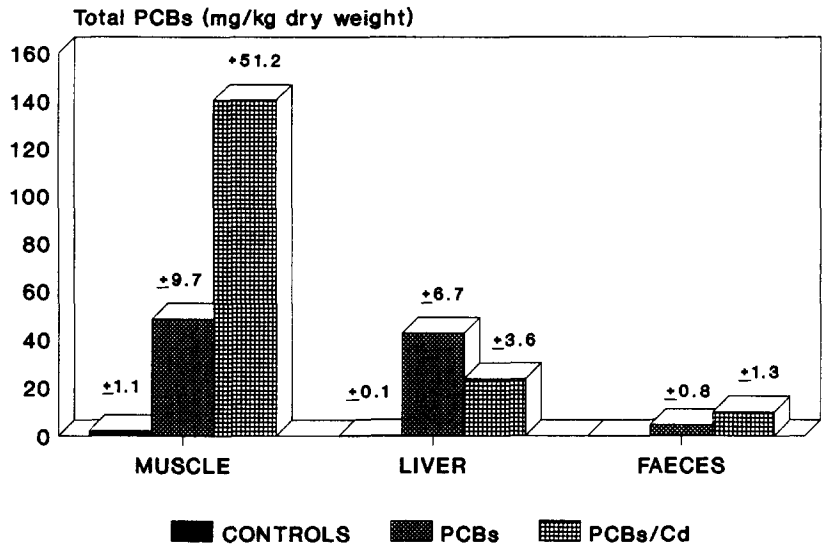
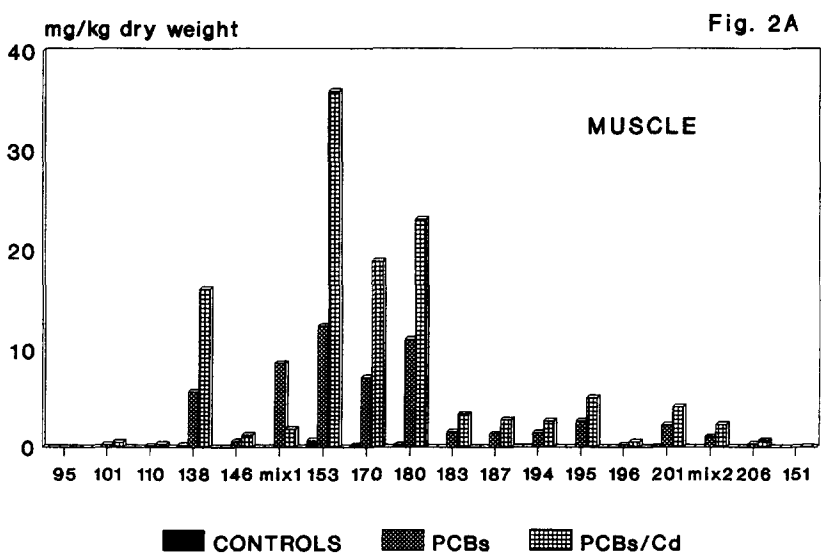
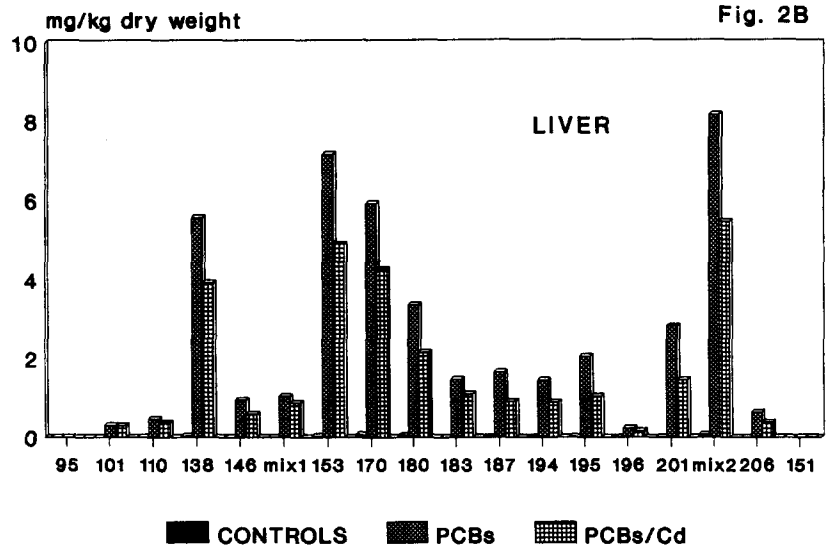


Figure 1. Total PCB concentrations (arithmetic mean ± S.D. on the top of the bars) in experimental quail





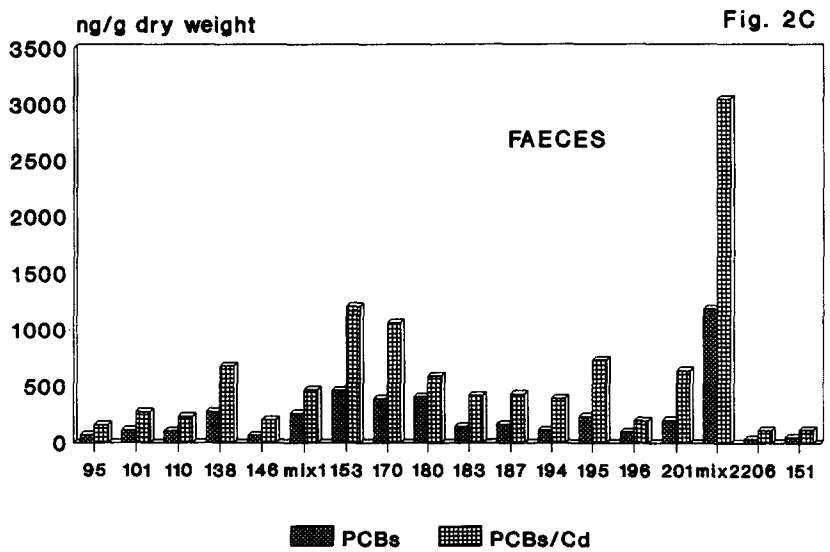


Figure 2. A,B,C - Average concentrations of various PCB congeners in muscle, liver and faeces of experimental quail

group they were 70 times higher than in controls ($p<0.0001$) and 3 times higher than in the PCB group ($p<0.001$).
There were no signficant qualitative differences between groups (Fig.2). The most frequent congeners were 138, 153, 170 and 180. Comparison with the congener composition of Arochlor 1260 showed that the percentages of congeners 138, 153, 170 and 180 were much higher in groups treated with PCBs and PCB/Cd. The proportions of congeners 183, 194, 195, 201 and 171-156-202 also increased whereas 95 and 151 disappeared altogether (Fig.2).
The total quantities of PCBs in liver were lower than in muscle in controls and in the PCB/Cd group, and were practically the same as in muscle in the PCB group (Fig.1). There were no substantial qualitative or quantitative differences between Cd/PCB and PCB groups. Of the congeners analyzed, 138, 153, 170 and 180 predominated, as in muscle, together with the 171-156-202 mixture (Fig.3).
The PCB group showed higher percentages of congeners 138, 146, 170, 180 183, 194, 195, 201, 206 and 171-156-202 than occur in Arochlor 1260. There were lower quantities of 180 and 196; 95 was undetectable. The same pattern was found in the PCB/Cd group (Table 2).

The concentrations of PCBs in the faeces of controls were below detection limits. In the PCB group, the total quantity of PCBs was about half that in the PCB/Cd group (Fig.1).

The congeners present in highest concentrations were 153, 170, 180 and 171-156-202 (Fig.4). Both treated groups showed a similar congener composition. The percentage of 171-156-202 was about 15 times higher than in Arochlor ($p<0.01$)(Table 2).

Environmental risk evaluation due to pollutants is often difficult to estrapolate from laboratory experiments. In nature, the maximum levels of cadmium and PCBs to which organisms are exposed are one or even more orders of magnitude lower than those utilized in this experiment. On the other hand, the time of exposure to contaminants is the whole lifetime of the animals, i.e. up to 10 years in the case of certain marine birds. Such long periods are impossible to reproduce in the lab. Every experimental model must necessarily be simplified. Accordingly in this experiment we drastically increased the inputs and decreased the time of exposure.
The treatment of quails with 100 ppm PCBs in the diet together with cadmium determined a macroscopic redistribution of PCBs in the organs. There was a dramatic increase in PCBs in muscle and faeces and at the same time a decrease in the liver. This type of interaction has not hitherto been described in laboratory experiments involving the simultaneous administration of Cd and PCBs in rats (Suzuki 1980) and mice (Andersen *et al.* 1985). Olsson *et al.* (1979) reported that exposure to low levels of PCBs increased the retention of cadmium naturally present in the diet of minks.
A previous study with quails (Leonzio *et al.* 1992) showed a change in levels of circulating fats in birds treated with Cd. A close relationship between triglycerides and tissue concentrations of organochlorine compounds (Aguilar 1985) has been reported in several studies. This metabolic parameter could be fundamental for explaining the increased accumulation of PCBs in muscle. A decrease in fat reserves in the liver after Cd treatment may be the reason for the decrease in accumulation of PCBs in this organ and the increase in muscle. PCBs are presumably mobilized together with fats from the liver, accumulating in muscle.

Fig. 3A

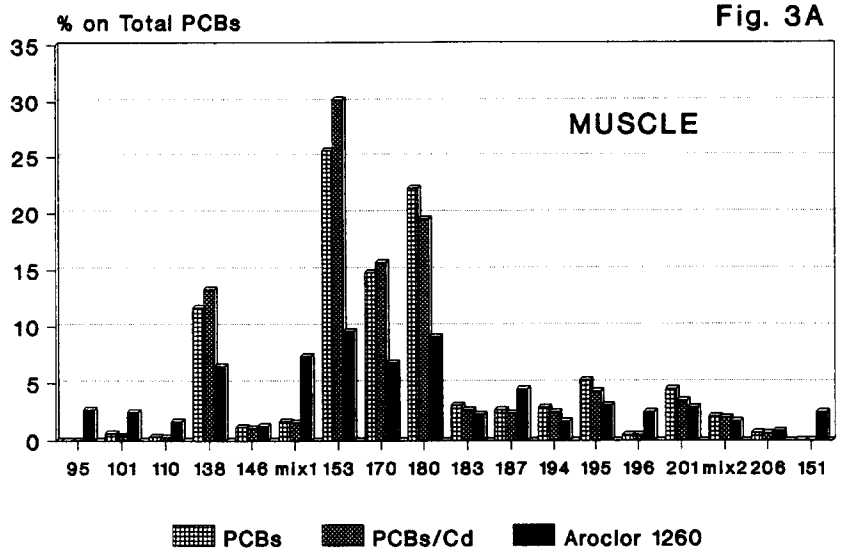
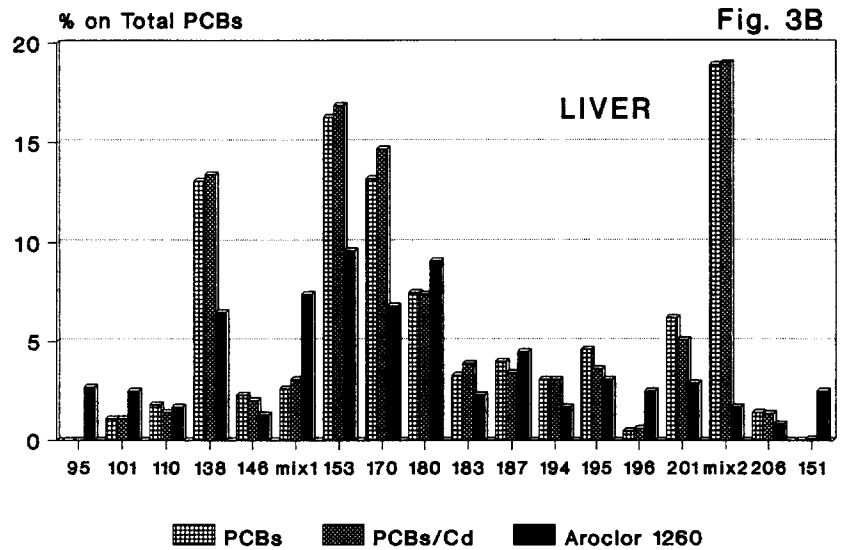

% on Total PCBs
35
30
25
20
15
10
5
0
MUSCLE
95 101 110 138 146 mix1 153 170 180 183 187 194 195 196 201 mix2 206 151
PCBs
PCBs/Cd
Aroclor 1260

Fig. 3B


% on Total PCBs
20
15
10
5
0
LIVER
95 101 110 138 146 mix1 153 170 180 183 187 194 195 196 201 mix2 206 151
PCBs
PCBs/Cd
Aroclor 1260

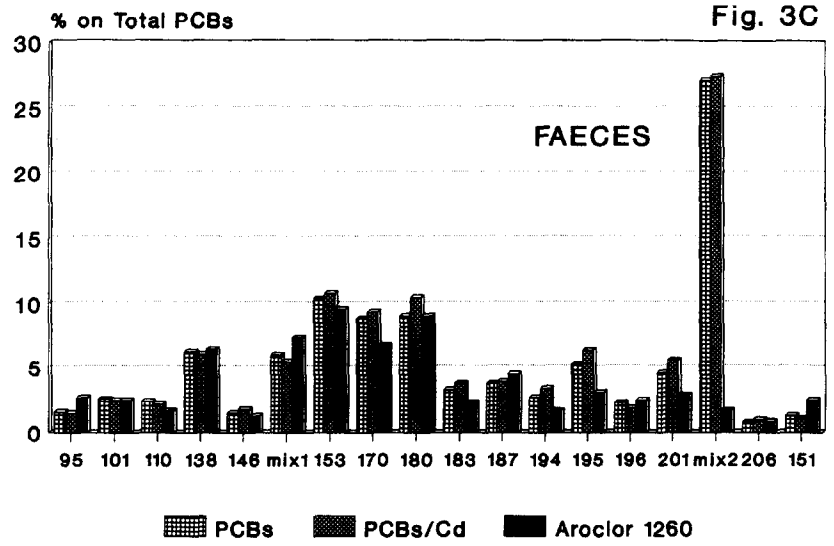


Figure 3. A,B,C - Percent composition of PCB congeners in experimental quail and in the commercial mixture Aroclor 1260

The increased excretion of PCBs after such treatment is probably related to this phenomenon.

The quantitative differences in accumulation of PCBs were not reflected in similar differences in congener composition in the treated groups. Lower chlorinated congeners were the only ones to be practically absent from accumulation organs like muscle and liver. In the faeces, these congeners reappeared in both groups. The metabolization of congeners like 153, 170, 180 and 138 by liver enzyme systems (MFO) often reported in the literature (Clarke 1986, Save *et al.* 1985) did not occur in this experiment. In fact there were no substantial differences between the configuration of the product administered (Arochlor 1260) and that in the different organs.

Data on wild birds shows marked differences in congener composition (Focardi *et al.*, 1988). These findings seem to sustain the hypothesis that PCBs persist in the food chain for a long time and are slowly metabolized at different trophic levels. Our data show that Cd at high levels interferes with high levels of dietary PCBs amplifying both quantitative and qualitative retention of congeners in muscle. In particular, there is an increase in congeners chlorinated in 2,4,5 position (e.g. 153, 138, 180 and 170) whic are more toxic and resistant to metabolic degradation.

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