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TOXICOLOGICAL INVESTIGATION OF POLYCHLORINATED BIPHENYL CONGENERS IN JAPANESE QUAIL: INDUCTION OF DRUG METABOLIZING ENZYMES AND PORPHYRIA

by

JOHN EDWARD ELLIOTT

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UNIVERSITY OF OTTAWA

For Christine, Kyle, Siobhan and Frazer

ABSTRACT

This thesis reports on investigations of the effects of Aroclor 1254 and four polychlorinated biphenyl (PCB) congeners, # 77 (3,3',4,4'-tetrachlorobiphenyl), # 105 (2,3,3',4,4'-pentachlorobiphenyl), # 126 (3,3',4,4',5-pentachlorobiphenyl) and # 153 (2,2',4,4',5,5'-hexachlorobiphenyl) on induction of polysubstrate monooxygenases and accumulation of porphyrins in liver and kidney of Japanese quail (*Coturnix c. japonica*).

Experiments were conducted to determine the relationship between the onset and severity of porphyria and polysubstrate monooxygenase activity in Japanese quail dosed with Aroclor 1254 or various PCB congeners. Female quail were dosed orally with the various PCBs and their livers and kidneys assayed for aldrin epoxidase (AE), aminopyrine n-demethylase(APND), 4-chlorobiphenyl hydroxylase (4-CBP) and ethoxyresorufin o-deethylase (EROD) activity. Those tissues were also analyzed by high performance liquid chromatography for concentrations of porphyrins and by gas chromatography-electron capture techniques for residues of the PCBs.

Chronic moderate dosing with Aroclor 1254 caused significant accumulation of hepatic porphyrins and significantly increased the activity of AE, APND, 4-CBP and EROD. Chronic dosing with PCB congener # 105 caused significant porphyrin accumulation and induction of APND, 4-CBP and EROD similar to that caused by Aroclor 1254, while congener # 126 caused mild but significant porphyrin accumulation and some minimal EROD induction. Chronic dosing with PCB # 153 caused slight porphyrin accumulation and induction of APND and EROD.

A single dose of Aroclor 1254 caused significant accumulation of hepatic and renal porphyrins, induction of EROD and APND and increase in liver weight. A single dose of # 126 caused extreme accumulation of highly carboxylated porphyrins in liver and kidney as well as induction of APND and EROD activity. A single high dose of congener # 77 caused no effects other than some minimal EROD induction, while the same dose of # 153 caused maximal APND and EROD induction and some porphyrin accumulation as well as liver enlargement. The batch of PCB 153 used was found to contain traces of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), which may account for the observed effects, especially of EROD induction. No PCDDs nor PCDFs were found in the batch of PCB 105. As trace amounts of PCDDs and PCDFs should be of less consequence to the toxicity of the non-ortho PCBs, # 77 and # 126, they were not analyzed.

RESUME

La présente recherche de thèse porte sur l'effet des biphényles polychlorés (BPCs) sur la caille japonnaise (*Coturnix c. japonica*). On a administré, par voie buccale, à des groupes de cailles femelles une dose non léthale d'un mélange commercial, Aroclor 1254, ou d'un de quatre biphényles polychorés a l'état pur. Les foies et les reins ont été analysés pour évaluer l'activité de certaines monooxygénases de polysubstrats (MOPS) et l'accumulation de porphyrines et de BPC.

Une dosage chronique avec l'Aroclor 1254 a causé une accumulation statistiquement significative de porphyrines dans le foie et a augmenté de façon significative l'activité des MOPS, de l'époxidase d'aldrine (EA), du N-diméthylase d'aminopyrine (NDAP), de l'hydroxylase de 4-chlorobiphényle (4-CBP) et du O-deethylase d'éthoxyrésorufine (EROD). Un dosage chronique avec le BPC no. 105 (2,3,3',4,4'-BPC) a augmenté la concentration des porphyrines ainsi que l'activité du NDAP du 4-CBP et de EROD de façon comparable à l'Aroclor 1254. Le BPC no. 126 (3,3',4,4',5-BPC) a, par contre, causé une faible augmentation de la concentration des porphyrines et de l'activité de EROD. Quant au BPC no. 153 (2,2',4,4',5,5'-BHC), il a produit une faible augmentation de la concentrationn des porphyrines et l'activité du NDAP et de EROD.

Un dosage unique d'Aroclor 1254 a produit une accumulation de porphyrines du foie et du rein; cette mème dose a augmenté l'activité de EROD et du NDAP et a causé un augmentation du poids du foie. Un dose unique de BPC no. 126 a causé l'accumulation extrème des porphyrines du foie et du rein ainsi que une augmentation de l'activité du NDAP et de EROD. Une dose plus élévée du BPC no. 77 (3,3',4,4'-BTC) n'a pas causé d'effet sauf une faible augmentation de l'activité de EROD. Une même dose du BPC no. 153 a, pour sa part, causé une forte augmentation de l'activité de NDAP et de EROD, une certaine accumulation de porphyrines et l'hypertrophie du foie. Le lot du BPC no. 153 a été analysé et on a détecté des traces de dibenzodioxines polychlorés (DDPC) et de dibenzofuranes polychlorés (DFPC). Le lot de BPC no. 105 ne contenait pas de DDPC ni de DFPC. Les lots de BPC no. 126 et no. 77 n'ont pas été analysés.

ACKNOWLEDGMENTS

The research presented in this thesis was conducted at the National Wildlife Research Center of the Canadian Wildlife Service. The successful completion of the work would not have been possible without the aid and cooperation of a number of people.

My research committee of David Peakall, Bernard Philogène and Tom Moon provided general guidance. I would also like to thank Sean Kennedy for his input to the project, also to Ross Norstrom for initial discussions.

I would especially like to thank the following:

Henry Won and Marek Zubic performed the PCB residue analyses. Mike Mulvihill provided the cleaned-up Aroclor 1254. Ross Norstrom and Mary Simon analyzed the PCB congeners for impurities. Suzanne Trudeau was of constant assistance in dealing with equipment and problems with biochemical methodology. Anne Macaulay, Bruce Dilabio, Jill Jensen, Pat Angehrn and Mary-Anne Marinic assisted in caring for birds and in the dissection and preparation of samples. Debbie Jeffrey was an invaluable teacher of laboratory technique, the philosophical approach to laboratory work and the handling of everything from live animals to final results. Ted Leighton provided histological analysis of thymus glands. Laird Shutt provided the quail. Glen Fox commented on earlier drafts of the thesis.

I thank Keith Marshall for his administrative and personal support. Finally, I wish to thank the Canadian Wildlife Service for financial support of the research and for allowing a leave of absence to complete it.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AE	aldrin epoxidase
ALA-S	aminolevulinic acid synthetase
AHH	arylhydrocarbon hydroxylase
APND	aminopyrine n-demethylase
4-CBP	4-chlorobiphenyl hydroxylase
COPRO	coproporphyrin
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
ECD	electron capture detector
ED50	effective dose - fifty
EROD	ethoxyresorufin o-deethylase
GC-EC	gas chromatography - electron capture
GC-MS	gas chromatography - mass spectrometry
HCB	hexachlorobenzene
HCP	highly carboxylated porphyrin (uro + hepta + hexa)
HPLC	high performance liquid chromatography
MC	methylcholanthrene
NADP	nicotinamide adenine dinucleotide phosphate
PB	phenobarbitol
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PHAH	polyhalogenated aromatic hydrocarbon
PROTO	protoporphyrin
PSMO	polysubstrate monooxygenase
QA	quality assurance
TCDD	tetrachlorodibenzo dioxin
TCDF	tetrachlorodibenzo furan
URO-D	uroporphyrinogen decarboxylase

1. INTRODUCTION

Environmental pollution by toxic substances has become a global problem with ecological, economic and political consequences. Polychlorinated biphenyls (PCBs) are among the substances which have aroused the most concern. Together with DDT, they most typify the environmental consequences of irresponsible use of the products of our technology.

Over 10^9 kg of PCBs were produced world wide during the past fifty years. During the 1970s, many countries imposed severe restrictions on production and use of PCBs. However, approximately a third of this quantity is estimated to have been lost to the environment and to exist in readily available and mobile environmental reservoirs (Tanabe, 1980). The amount released to the environment is gradually being redistributed from highly contaminated to less contaminated areas. Hazardous levels continue in some areas and little is known of the long term threat from stable or increasing PCB contamination of ecosystems.

In order to assess the environmental threat from PCBs, their complex toxicity must be better understood. Much progress has been made in studying the effects of PCBs on laboratory mammals; however, little is known of the relevance of this work for other species, including birds. This thesis attempts to address the sublethal effects of both a commercial mixture of PCBs and some selected congeners on an avian laboratory model, the Japanese quail.

1.1 Structure, Properties and Uses of PCBs

PCBs are complex mixtures of congeners formed during the chlorination of the biphenyl molecule. A total of 209 structures are possible with varying substitutions of from one to ten chlorine atoms on the biphenyl rings. Ballschmiter & Zell (1980) devloped a shorthand numbering system for individual congeners. That system is employed in this thesis and the Appendix contains a table which cross-references the structural formula with the Ballschmiter - Zell number. Figure 1.1 shows how the biphenyl ring system is numbered for determining the structural name.

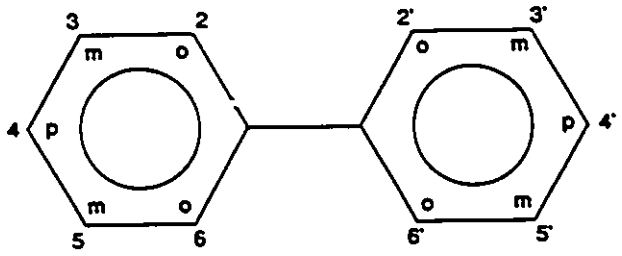


Fig. 1.1 Structure of polychlorinated biphenyls showing how the biphenyl rings are numbered for determining the structural name.

PCBs were formulated and marketed according to their total chlorine content. Monsanto Corp. produced PCB mixtures under the trade names Aroclor 1242, 1248, 1254 and 1260, which contained 42%, 48%, 54% and 60% chlorine, by weight, respectively. The chlorine content of the mixture roughly corresponds to the degree of chlorination of the biphenyl nucleus, i.e. Aroclor 1254 contains mainly tetra to hexa isomers while 1260 contains mainly penta to hexa isomers.

PCBs were used for a variety of purposes which can be divided into "closed circuit" uses such as in electrical transformers and capacitors and in heat transfer and hydraulic systems and into "open uses" such as the formulation of lubricating and cutting oils, pesticides, plastics, paints, inks, adhesives, etc. More than 1 billion (10^9) kg of PCBs were produced worldwide (Tanabe, 1988). Until 1977, over 90 % of the production was in the U.S.A., after that time production switched to Europe and Japan. It has been estimated that about 40 million kg of PCBs were imported into Canada; a recent inventory accounted for some 24 million kg and assumed that the remaining 16 million kg had already been lost to the Canadian environment (Environment Canada, 1985).

1.2 Environmental Contamination by PCBs

The polychlorinated biphenyls (PCBs) were first identified as environmental contaminants in Sweden (Anonymous, 1966). Since then, numerous studies have reported the presence of PCBs throughout the global ecosystem (Risebrough et al., 1968; Ballschmiter et al. 1981). Those findings contributed to a decision to voluntarily halt all "open" uses of PCBs in 1971 while "closed" uses as dielectric fluids were permitted in North America until 1977.

There is a great deal of published and unpublished information on the contamination of the global environment by PCBs (see reviews: Waid, 1987; Safe, 1987). A number of studies have shown that total PCB concentrations have decreased in biota of highly contaminated environments such as the Baltic Sea (Olsen & Reutergardh, 1986; Passivirta et al., 1980) and the Great Lakes (Mineau et al., 1984). Most of the declines in total PCBs in those areas took place during the initial period after the bans on PCB use, i.e. the late 1970s to the

early 1980s. Subsequently, it appears that the declines have largely leveled off. In the less contaminated regions that have been studied, including the upper Great Lakes, the St. Lawrence River and in the open ocean, PCB levels in biota changed very little during the same time frame (Addison et al., 1984; Stout, 1987; Pearce et al., 1989). Tanabe (1988) recently examined the global situation regarding PCB contamination. He concluded that the amount of PCBs still in use and in landfills, storage dumps, etc. is more than double the amount that has already been lost to the environment. Therefore, if more stringent measures are not taken to destroy those PCBs or remove them permanently from circulation, then significant input of PCBs to the environment will continue for many years.

Most of the information on environmental trends of PCBs is based on estimates of total amounts. Very little has been reported on the fate of individual congeners, in part because synthesis and retention time calculation for all 209 PCB congeners was only quite recently reported (Mullin et al., 1984). In the Hudson River sediments, Brown et al. (1987) reported selective dechlorination of PCB congeners which tended to favour loss of those congeners, including # 105, which are of more toxic concern. This phenomenon has not been reported from other, especially less polluted, locations.

1.3 Toxicity of PCBs

1.3.1 Commercial Mixtures

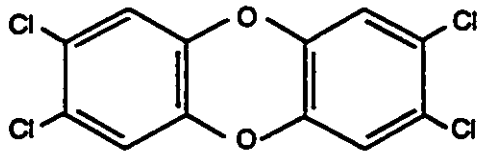
When compared to many pesticides, PCB mixtures are not acutely toxic to birds. Acute LD50s of Aroclors 1254 and 1232 to Bobwhite Quail are 340 and 1380 mg/kg respectively (Heath et al. 1972). By way of

comparison, the acute LD50 for endrin, an organochlorine insecticide still used in the U.S., is 1.2 mg/kg in California Quail and the acute LD50 for diazinon, a widely used organophosphate insecticide, is 3.5 mg/kg in mallard ducks (Hudson et al., 1984). In general, for bird species, acute toxicity of PCB mixtures increases with higher chlorine content within the range Aroclor 1221, 1232, 1242, 1248 and 1254; however, the higher chlorinated 1260, 1262 and 1268 are less toxic than 1254 (Heath et al., 1972).

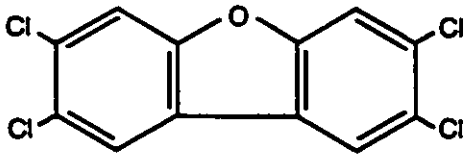
On the other hand, some mammals especially ranch mink are acutely sensitive to the toxic effects of PCBs. A diet containing 3.6 ppm of Aroclor 1254 caused 100 % mortality within 105 days in a group of experimental mink, while mink on 0.64 ppm diet failed to produce any viable young (Platanow & Karstad, 1973).

There are many chronic sub-lethal effects of PCBs and these have been reviewed a number of times (Kimbrough, 1974; McConnell, 198C; Parkinson & Safe, 1981; Safe, 1984). A suite of toxic symptoms are common to most species studied. These include: dermal lesions and acne, weight loss beyond that caused by lower food consumption (wasting syndrome), immunotoxicity involving thymic atrophy, liver enlargement and other signs of hepatotoxicity such as porphyria, induction of hepatic and extra-hepatic drug-metabolizing enzymes and reproductive toxicity. There is, however, considerable variation among species in sensitivity to chronic PCB exposure. Among birds, chickens are particularly sensitive; Aroclor 1254 concentrations greater than 5 mg/kg in eggs will reduce hatchability (Platonow & Reinhart, 1973).

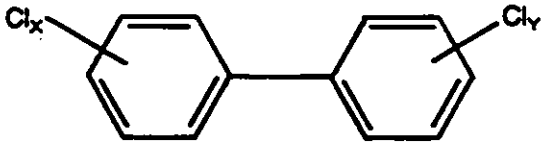
Fig. 1.2 Structures of 2378-TCDD, 2378-TCDF and the PCBs.



2,3,7,8 - Tetrachlorodibenzo-p-dioxin



2,3,7,8 - Tetrachlorodibenzofuran



Polychlorinated Biphenyls

1.3.2 Congener Toxicity Patterns

The toxic symptoms caused by PCB mixtures are also common to the PCDDs (polychlorinated dibenzo dioxins) and the PCDFs (polychlorinated dibenzo furans). The similarities in structure are shown in Fig. 1.2.

The toxicity of the individual compounds among this group of related polyhalogenated aromatic hydrocarbons (PHAHs) varies greatly with the molecular structure. The most toxic PHAH is 2,3,7,8-TCDD which is often used as a model for the toxicity of the related compounds. The most toxic furan and biphenyl congeners all exhibit a similar structure to 2,3,7,8-TCDD. There have been a number of attempts to systematically study the effects of the PHAHs in order to elucidate possible structure activity relationships (Poland et al., 1983; Safe, 1984).

Those PCBs which are similar in structure to TCDD, i.e. which contain no ortho, a para and at least 1 meta chlorine on each ring, i.e. #'s 77, 126 and 169 are all potent in vitro P-450-MC inducers. This similarity in structure and effects is believed to be mediated by initial binding of the polyhalogenated aromatic to a cytosolic protein, referred to as the Ah receptor. This inducer-receptor complex is then translocated into the nucleus, where it activates a variety of structural genes including those coding for the polysubstrate monooxygenase (PSMO) enzymes, especially those of the cytochrome P-450 class (Nebert et al., 1981).

1.3.2.1 Induction of Polysubstrate monooxygenases

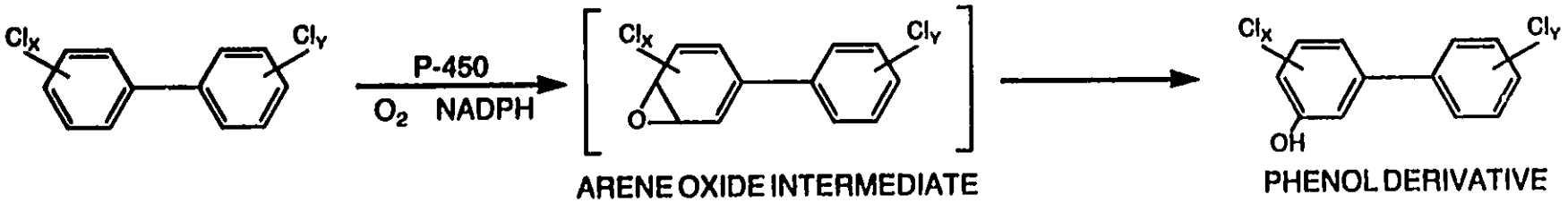
Induction of polysubstrate monooxygenases by PCBs and other PHAHs is one of the most sensitive responses to this group of chemicals. The PSMOs are enzymes associated with the endoplasmic reticulum or microsomal fraction of a number of tissues, particularly the liver.

They appear to function primarily in the metabolism of foreign and some endogenous compounds. The reactions catalyzed by the PSMOs are NADPH dependent and involved uptake of two electrons from NADPH, the reduction of one oxygen atom to water and insertion of the other oxygen atom into the substrate, such as shown in the metabolism of a PCB compound in Fig. 1.3. The terminal oxygenases in this system are the cytochrome P-450 family of heme-containing proteins (Macdonald, 1984; Ioannides & Parke, 1987). The cytochromes P-450 can be divided into a number of distinct gene families (Nebert & Gonzalez, 1985). The most studied are those induced by phenobaritol and the polyaromatic hydrocarbons such as methylcholanthrene. There are a number of nomenclature systems used to designate cytochrome-P-450 groups. This thesis uses the designation P-450-PB to refer to those enzymes induced by phenobarbitol and P-450-MC to those induced by methylcholanthrene in the rat.

PCBs such as # 153, which have 2 ortho-substituents are classified as P-450-PB inducers in rats as they evoke a response similar to dosing with phenobarbital. They are often major components of commercial PCB mixtures. The PCB congeners such as # 126 which resemble TCDD are classified as P-450-MC inducers as they evoke a response similar to dosing with methylcholanthrene. These congeners are generally present in low amounts in PCB commercial mixtures (Kannan et al., 1988). The third class of PCB compounds, the mixed inducers, include the mono- and di-ortho compounds with a 3,4,3',4'- pattern and evoke both a P-450-PB and P-450-MC type response. Some of these compounds, such as # 105 are major components of commercial PCB mixtures.

Fig. 1.3 Mixed function oxidase mediated metabolism of a PCB congener to its hydroxylated derivative.

METABOLISM OF PCBs



ARENE OXIDE INTERMEDIATE

PHENOL DERIVATIVE

1.3.2.2 PCBs and Porphyria

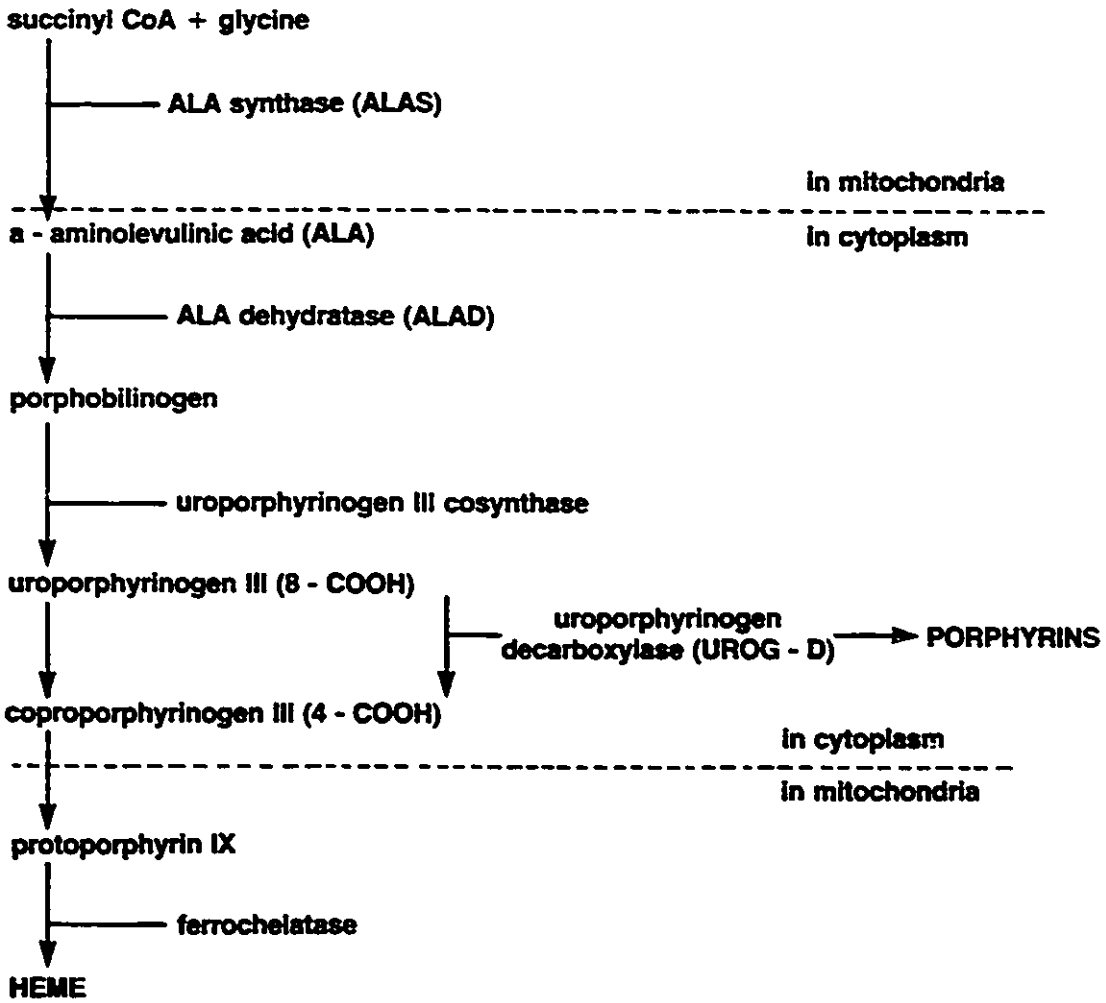
PCBs and other PHAHs have been shown to cause hepatic porphyria in laboratory mammals and birds in a number of studies (Marks, 1985). Porphyria is a disorder in which inborn or chemical-induced impairment of heme biosynthesis results in an alteration in the size and/or composition of the porphyrin pool (Meyer & Schmid, 1978).

Heme biosynthesis is centered in erythroid cells where heme is required for hemoglobin production and in the liver, where heme is used in the production of various hemoproteins, particularly cytochrome P-450. Figure 1.4 shows the heme biosynthetic pathway.

Fig. 1.5 shows the basic porphyrinogen and porphyrin structures. Under normal conditions only small amounts of porphyrins other than protoporphyrin IX are produced in cells. However, if the pathway is altered then the size and composition of the porphyrin pools can change, resulting in porphyria (Marks, 1985). Porphyrins and precursors can accumulate in cells and elevated amounts will also be present in feces and urine. Exposure to certain PHAHs such as HCB, some PCBs and PBBs and 2,3,7,8-TCDD in addition to aflatoxin B_1, ethanol and therapeutic steroids, results in the hepatic accumulation of highly carboxylated porphyrins (uroporphyrin, heptacarboxylic and hexacarboxylic porphyrins or HCPs) in susceptible strains, species and in genetically predisposed individuals (Marks, 1985; Meyer & Schmid, 1978).

A number of groups have used an avian cell culture system prepared from chick embryo hepatocytes to examine the structure activity relationships among PCB congeners and their porphyrinogenicity (Sano *et al.*, 1985; Sassa *et al.*, 1986). Those studies suggest that the diortho (2,2'-) substituted PCBs do not cause significant porphyrin accumulation

THE HEPATIC SYNTHESIS OF HEME



in vitro. The *in vitro* structure activity relationships among PCBs and their porphyrinogenicity also indicates that the effect is mediated at the *Ah* locus. By the use of specific inhibitors of P-450 enzymes, such as piperonyl butoxide, some investigators have examined the role of P-450-MC induction in HCP accumulation in chick embryo liver cell culture (Debets *et al.*, 1980; Sinclair *et al.*, 1987).

Fig. 1.5 Structure of porphyrins and porphyrinogens.

In vivo studies in rats indicate that only P-450-MC or mixed inducers cause porphyria (Stonard & Grieg, 1976). However, in chicks, supposed pure P-450-PB type inducers, including # 153, increased porphyrin levels (Goldstein *et al.*, 1976), while 2,2',4,4'-TCB caused significant prophyrin accumulation in Japanese quail (Miranda *et al.*, 1987).

Of the chemicals which appear to cause elevated tissue HCPs, only the PHAHs are significant environmental contaminants. Therefore, measurement of highly-carboxylated porphyrin levels shows some promise as a "bio-effects marker" for certain PHAHs (Marks, 1985; Fox *et al.*, 1988). Derangements of certain heme biosynthetic functions are already

used as indicators of exposure to other environmental contaminants such as the heavy metal, lead. Inhibition of aminolevulinic dehydratase (ALA-D), a key enzyme in the heme biosynthetic pathway, results from lead poisoning and is widely used an index of short-term lead exposure (Hernberg et al., 1970).

However, some basic questions need resolving before porphyrin levels can be used as an effective indicator of biological exposure and possibly effects of PHAHs. Further work on other avian species is necessary to determine which PHAH compounds cause porphyria, to examine the dose response and temporal development of the disorder and also to consider its relationship with PSMO induction and other signs of toxicity.

1.3.3 Environmental effects on biota

Much has been written on the laboratory effects of PCBs; however, less is known of their effects on the environment. Hansen (1987) has recently reviewed the literature on the environmental effects of PCBs.

Two recent field studies in particular prompted the work undertaken in this thesis. In the Green Bay area of Lake Michigan, poor reproductive success of Forster's Terns (*Sterna forsteri*) (Hoffman et al., 1987; Kubiak et al., 1989) has been linked to contamination by PCB congeners, in particular, # 126 and # 105. Another recent study showed that Herring gulls (*Larus argentatus*) from the Great Lakes had hepatic porphryia which was associated with elevated levels of PCBs and other PHAHs (Fox et al., 1988). In addition, Tanabe et al. (1987) recently reported elevated levels of PCB #s 77 and 126 in marine mammals from the north Pacific.

1.4 Thesis Overview

This thesis presents the results of two experiments designed to study the effects of PCBs on induction of polysubstrate monooxygenases and accumulation of porphyrins in Japanese Quail. Details of materials and methods used in both experiments are in Chapter 2. The effects of dosing with the individual congeners, 153, 105 and 126 for up to 8 weeks and of Aroclor 1254 for up to 12 weeks are in Chapter 3. Chapter 4 presents the findings of an experiment to determine the effects of a single oral dose of Aroclor or of three PCB congeners, #s 153, 126, 77. Figures and/or summary tables are incorporated into the text of each chapter. Detailed data tables which include the statistical significance of pairwise comparison of dosed and control groups, are at the end of each chapter.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Selection of an animal model

Japanese quail were selected as an experimental animal as they have been used in a variety of studies of PHAH-induced porphyria (see for example Vos et al. 1971, Carpenter et al., 1985, Miranda et al., 1986). They are readily available from commercial suppliers, are relatively easy to feed and house and therefore provide a good model for toxicology studies of birds.

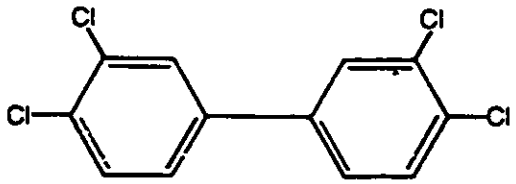
2.1.2 Selection of PCBs for experimentation

Aroclor 1254 was used as a representative PCB mixture primarily because it has been shown to be the main form of PCBs present in samples of Canadian wildlife (Norstrom, 1988). It also has been shown to contain the individual congeners used in the study (Kannan et al., 1988). Aroclor 1254 is also commonly used as a representative mixed inducer in pharmacological studies.

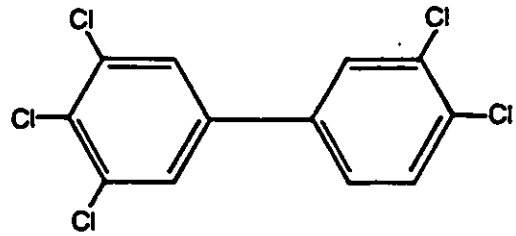
The structure of the PCBs used in this study are in Fig. 2.1. PCB # 126 is the most potent AHH inducer and the most toxic of the non-*ortho* or coplanar PCBs in rats (Safe, 1984) and in chick embryos (Brunstrom & Andersson, 1988). PCB 77 is a less toxic than PCB 126 in rats and chick embryos, but it has been reported to cause porphyria in chick embryo liver cell culture and in male Japanese quail (Miranda et al., 1987).

Although, the non-*ortho* substituted PCBs appear to be the most toxic congeners, those compounds with a similar 3,3',4,4'- substitution which are also substituted at one or more *ortho* positions have also been found to be toxic. They elicit both P-450-PB and P-450-MC type

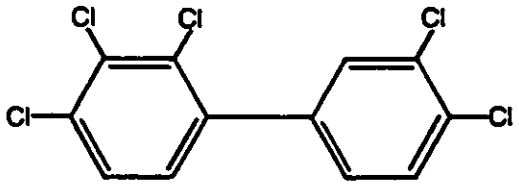
Fig. 2.1 Structure of four PCB congeners



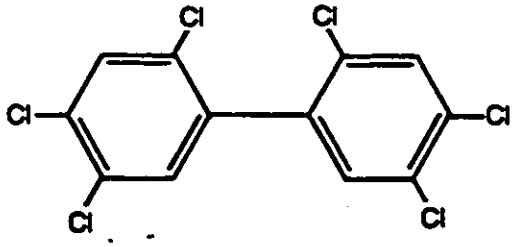
77
3,3',4,4'-Tetra



126
3,3',4,4',5-Penta



105
2,3,3',4,4'-Penta



153
2,2',4,4',5,5'-Hexa

response. They are also significant components in Aroclor mixtures and constitute some of the major PCB peaks present in wildlife samples (Norstrom, 1988). Among the mixed inducers, 2,3,3',4,4'-pentachlorobiphenyl (# 105) is a particularly interesting isomer. Although not as readily accumulated in widlife as some other mixed inducers such as 2,2',3,4,4',5'-hexachlorobiphenyl (# 138), PCB 105 is a much more potent inducer of AHH, at least in rats (Leece et al., 1985).

The other class of PCB congeners are the P-450-PB type inducers. Congener 2,2',4,4',5,5'-hexachlorobiphenyl (# 153) is one of the more interesting compounds from this group, primarily because it is the dominant PCB congener in wildlife samples (Norstrom, 1988). Although, widely considered to be less toxic and a strict P-450-PB type inducer, there are reports of this congener causing porphyria in chicks (Goldstein et al., 1976) and chick-embryo cell culture (Zelt, 1980).

2.1.3 Chemicals

Aroclor 1254 was purchased from Monsanto Chemicals. It was cleaned up on a florisil column in order to remove possible dibenzofuran contaminants (Bowes et al., 1975). Approximately one gram of the mixture was dissolved in 40 ml of hexane and added to a glass column (3.2 cm diameter, 45 cm long) containing 150 g of florisil (deactivated with 1.2 % water), topped with 2.5 cm of sodium sulphate and prewetted with hexane. The mixture was then eluted with 750 ml hexane (fraction 1) then three times with 500 ml of 85 % hexane : 15 % methylene chloride (fractions 2,3,4) and finally with 750 ml of methylene chloride (fraction 5). The fractions were then analyzed by GC/ECD to test for loss of 'coplanar' PCB compounds, in particular #s 77, 126 and 169, during the clean-up. As no significant amounts of those congeners were

found in the successive fractions, only the hexane fraction (minus the hexane) was used for preparation of Aroclor 1254 for dosing.

PCB congeners, 2,2′,4,4′,5,5′-HCB (153) (95 %), 2,3,3′,4,4′-PCB (105) (95 %) and 3,3′,4,4′,5-PCB (126) (98 %) were purchased from Wellington Laboratories (Guelph, Ont.). PCB congener 3,3′,4,4′-TCB (77) (99 %) was purchased from Ultra Scientific (Hope, R.I.).

Porphyrins were from Porphyrin Products (Logan, Utah).

Sources of chemicals for PSMO assays were as follows: ethoxyresorufin from Pierce Chemicals, resorufin and aminopyrine from Aldrich Chemicals, aldrin and dieldrin from Shell Chemicals, 4-chlorobiphenyl and 4-chlorobiphenylol from Ultra Scientific (Hope, R.I.).

Sepharose Cl-2B was bought from Pharmacia Fine Chemicals (Etobicoke, Ontario). All other chemicals and reagents were purchased from Sigma Scientific (St. Louis, Mo.), Fisher Scientific (Ottawa, Ont.) or CanLab (Ottawa, Ont.).

2.1.4 Birds

Eight-week old female Japanese quail weighing between 200 and 225 grams were obtained from Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec. Birds were kept in wire mesh cage units (40 cm x 18 cm x 24 cm), two birds per cage. The experiment was conducted in an indoor aviary with a photoperiod (fluorescent lighting) of 13 hours of daylight and 11 hours of darkness. The room temperature was 21 $\pm$6.5 ^{o}C. Humidity was not measured. Water and food (CO-OP Turkey Starter, 26 % protein, 0.0125 % amprolium) were supplied ad libutum. The quail were kept a minimum of 1 week prior to dosing.

2.2 Methods

2.2.1 Safety Precautions

The following measures were taken to avoid contamination of the laboratories and animals holding facilities by PCBs:

1) All handling of chemicals was done under a fumehood and while wearing gloves. The work was always done over several sheets of aluminum foil.

2) All disposable materials used for the preparation of doses were discarded in special bins designed for disposal of high hazard chlorinated wastes. The bins were kept in a locked and monitored area until removed by a waste management contractor.

3) Animals were kept in a facility separate from the laboratories and offices. Negative air pressure was maintained in the room by constant venting of air to the outside. Feces and carcasses were triple bagged, sealed in cardboard boxes and collected for land fill disposal.

2.2.2 Dosing of birds

Doses were prepared by first dissolving the PCBs in a minimum of hexane (Aroclor 1254) or benzene (congeners), adding the required amount of corn oil and then removing the hexane or benzene by evaporation under nitrogen or by rotoevaporation over low heat. Concentrated stock solutions were prepared in corn oil and then diluted to obtain the required concentrations, resulting in a final dose of 220 microlitres of cornoil per bird. PCB 77 was not adequately soluble in acetone, hexane, benzene, iso-octane or toluene. Therefore, doses for # 77 were prepared as a suspension in corn oil with constant vortexing to maintain a milky suspension. For the chronic study, doses were constantly adjusted throughout the study to maintain a constant dose per kg body weight.

The dose was administered orally in one Number 4 gelatin capsule

for the chronic studies and in two No. 4 capsules for the acute study. Control birds were dosed with one or two capsules containing only cornoil. Dosing took place three time a week (Monday, Wednesday and Friday) for the duration of each chronic study. A single dose was given for the acute study.

2.2.3 Sacrifice of birds, tissue processing and storage

The birds were fasted for 12 hours prior to sacrifice and were killed by decapitation. Livers and kidneys were removed, separated into left and right lobes, weighed, put into plastic bags, heat-sealed and placed immediately in liquid nitrogen. Samples for PSMO analysis were kept in liquid nitrogen, samples for porphyrin analysis were transferred to a freezer (-72 ^{o}C), samples for chemical analysis were stored in a freezer (-40 ^{o}C) until analyzed. Individual nodes of thymus glands were carefully removed from surrounding tissue, placed on weighing paper, weighed and then stored in formalin. The whole bursa of Fabricus was removed, weighed and stored in formalin. All work was carried out according to the guidelines of the Canada Council on Animal Care.

2.2.4 Preparation of Microsomes

Livers were thawed overnight on ice, weighed and then chopped with buffer solution (0.1 M $NaKPO_4$, pH 7.4). Work was done on ice. Samples were homogenized in chilled buffer using a Potter-Elvehjelm homogenizer. The homogenate was centrifuged for 10 min. at 12,000 g. Microsomes were isolated by gel filtration according to the method of Pyykko (1983). Samples were placed onto a 16 ml bed of Sepharose gel in 1 x 30 cm glass columns and eluted with 8 ml of buffer. Eluate was collected in 1.2 ml cryovials and stored in liquid nitrogen until analysis.

2.2.5 Protein Assay

Microsomal protein was determined by the Lowry method as modified by Peterson (1977). The protein concentration was calculated using a linear standard curve (Stauffer, 1975).

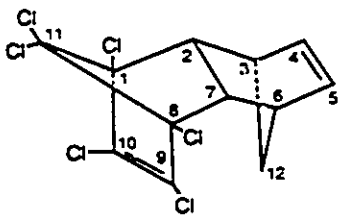
2.2.6 Polysubstrate Monooxygenase Enzyme Assays

The basic chemical conversions for each of the PSMO assays used are shown in Fig. 2.2.

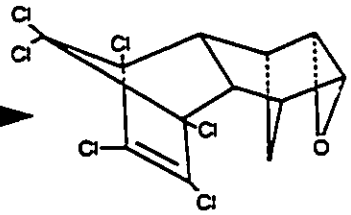
Aldrin epoxidase activity was measured by determining the amount of dieldrin formed using gas chromatographic analysis (Krieger & Wilkinson, 1969). An incubation mixture was prepared from 2.3 umol of glucose-6-phosphate, 1.5 units of glucose-6-phosphate dehydrogenase, 0.5 umol of NADP, 0.75 mg of microsomal protein and 0.1 umol of aldrin substrate. The mixture was incubated at 42° C for 15 min and 5 ml of hexane was added to stop the reaction. The mixture was centrifuged and the organic layer was removed and analyzed by GC-EC for formation of dieldrin. Analysis was performed using a Hewlett-Packard GC 5730A.

The activity of **aminopyrine n-demethylase** was measured by determining the amount of formaldehyde formed by means of the Hantzsch reaction (Lockwood & Houston, 1979; Bien *et al.*, 1981). A reaction mixture was prepared with 1.0 mg of protein, 100 ul of 60 mM aminopyrine, and a NADPH regenerating system consisting of 5 umol sodium isocitrate, 0.4 umol $NADP^+$, 5 umol $MgCl_2$ and 0.65 units of isocitrate dehydrogenase. The mixture was then made up to 2 ml volume with 0.1M NaKPO4 buffer. Tubes were incubated for 10 min at 37 ° C and the reaction stopped by addition of 0.175 ml 40 % TCA. The mixture was then centrifuged for 10 min. 1.0 ml of Nash reagent was then added to 1.0 ml aliquots of supernatant and heated for 40 min at 37 °C. The absorbance

Fig. 2.2 Structural representation of chemical changes used to assay for polysubstrate monooxygenase activity.

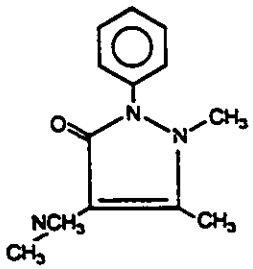


Aldrin

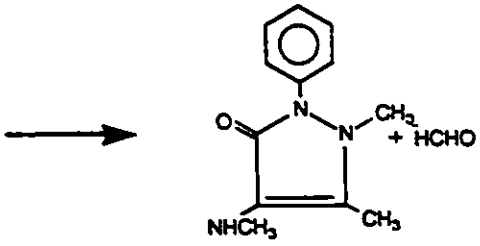


Dieldrin

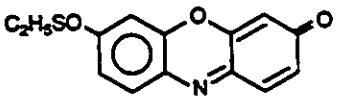
a)



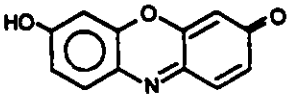
Aminopyrine



b)

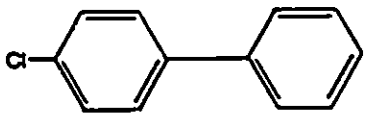


Ethoxyresorufin

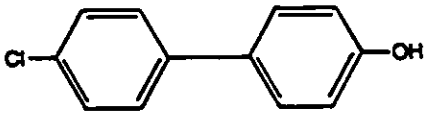


Resorufin

c)



4-chlorobiphenyl



4'-chloro-4-biphenylol

d)

was read at 412 nm against a reference of distilled water on a Beckman DU 5 spectrophotometer.

The activity of **7-ethoxyresorufin-o-deethylase** was determined by the method described in Nebert & Gelboin (1968). A reaction mixture was prepared with 125 nmol NaKPO4 buffer, 1.9 nmol 7-ethoxyresorufin, 0.1 mg microsomal protein and 125 ul of the NADPH regenerating system described above. The mixture was incubated for 10 min at 37 °C and the reaction stopped by addition of 2.5 ml methanol. Precipitated protein was removed by centrifugation and the fluorescence of resorufin measured in the supernatant at 585 nm using an excitation wavelength of 550 nm on a Turner Model 430 spectro fluorometer.

The activity of **4-chlorobiphenyl hydroxylase** was measured by HPLC determination of the amount of 4-chlorobiphenylol produced (Parkinson et al. 1980b). An incubation mixture was prepared from 8 umol of glucose-6-phosphate, 1.5 units of glucose-6-phosphate dehydrogenase, 0.5 umol NADP, 0.5 mg protein and 100 umol of 4-chlorobiphenyl substrate (in methanol:tween 80, 95:5). The mixture was incubated for 20 minutes at 42°C and the reaction stopped by addition of 1.0 M acetate buffer, pH 4.6. The mixture was then extracted three times with hexane and evaporated under nitrogen until a 500 ul volume of methanol remained. The sample was filtered through 0.45 um HV filters and stored at 4°C (maximum 24 hours) until analyzed with a Perkin-Elmer Series 4 HPLC with a Perkin-Elmer UV detector LC 85B, equipped with a 5,Rp 18 Brownlee guard column and a 5 ODS Partisil, 3 RAC II separating column. The mobile phase was methanol:water (88:12) with a flow rate of 1.0 ml/min.

2.2.7 Quality assurance procedures

All PSMO assays were performed in duplicate and the mean of the two

measurements taken as the value. In order to assure the accuracy of PSMO results an internal reference material (QA pool) was prepared as follows. Five female Japanese quail were dosed with Aroclor 1254 and sacrificed after 5 days as described above. The livers were then pooled, homogenized microsomes prepared and the 1.2 ml samples aliquotted and stored as described. For each assay, five samples of the QA pool were analyzed in duplicate using the methods described below. The mean of the five samples then constituted a 'control value' for that assay. At least one sample from the QA pool was then included in all PSMO analyses made for this study. Criteria for rejecting any batch of samples was set according to Henry et al. (1977) i.e. the results for the positive control must fall within 1 standard deviation of the established control value.

2.2.8 Tissue analysis for PCBs

PCB concentrations in livers and kidneys were determined by methods described in Peakall et al. (1986). Liver samples (1.5 to 2.0 grams) were ground with sodium sulphate, poured into a chromatography column and eluted with hexane. Extracts were reduced to 5 ml by rotary evaporation then fractionated by florisil chromatography using hexane and dichloromethane. Fractions were analyzed by gas chromatography with an electron capture detector. The amount of Aroclor 1254 present in the samples was calculated by peak height comparison using the peak for PCB 118. Congeners were quantified using commercial standards and verified with standards prepared from dosing material.

2.2.9 Analysis of PCB congeners for PCDDs and PCDFs

PCB congeners # 153 and # 105 were analyzed for trace PCDD and PCDF contamination by GC-MS according to methods described in Norstrom et al.

(1986). Briefly, 1 mg of the PCB material was accurately weighed and dissolved in 5 ml hexane. The solution was then loaded on the top of a large glass column packed with anhydrous sodium sulphate and extracted with 300 ml of dichloromethane/hexane (1:1). Volume of the extract was reduced to 5 ml and subjected to cleanup by gel-permeation/carbon column/florisil column/alumina column chromatography. The sample was then analyzed on a Hewlett-Packard 5987B GC-MS.

2.2.10 Porphyrin Analysis

Porphyrins were analyzed by HPLC according to the method of Kennedy et al. (1986a). About 0.5 grams of tissue were accurately weighed; 10 ml of 0.9 N perchloric acid/methanol, 50/50 was added to the tissue and homogenized for 5-10 seconds in a Ultra-Turrax (Janke and Kunkel) homogenizer. The mixture was then centrifuged (2500 rpm, 8 min.) and the supernatant added to 30 ml of distilled water. A SEP-PAK C_{18} cartridge (Waters, Mississauga) was prepared for porphyrin concentration by wetting first with methanol (10 ml) and then with water (15 ml). The porphyrins were then concentrated at the head of the cartridge by flushing the extract through the cartridge with a 50 ml syringe. The extraction procedure was then repeated and the extracts were combined. Porphyrins were eluted from the cartridge with 1.5 ml methanol and the eluate filtered through a 0.45 um membrane filter. HPLC analysis was by reverse phase gradient elution of porphyrins using a sodium phosphate/methanol mobile phase at a flow rate of 2.5 ml/min and a Perkin Elmer 3 cm long C_{18} column(3 um particle size). High-performance-liquid-chromatography was carried out with a Perkin-Elmer Series 4 liquid chromatograph equipped with a Rheodyne 7135 loop injector valve (5 ul capacity). The detector used was a Perkin-Elmer

LS-4 fluorescence spectrophotometer with the excitation wavelength set at 365 or 400 nm (15 nm slit) and the emission wavelength set at 624 nm (20 nm slit). Porphyrin peaks were quantified by comparison of peak heights with those of external standard solutions of uroporphyrin III, coproporphyrin III and protoporphyrin IX respectively. Heptacarboxylic acid porphyrin was quantified with the porphyrin standard.

2.2.11 Histology

The thymus glands were dissected from the birds, weighed and placed into 10 % buffered formalin. Thymuses from the two -week congener study and the four-week Aroclor study were sent to the University of Saskatchewan, where they were sectioned and examined under light microscope by Dr. T. Leighton for histological differences between dosed and control birds.

2.2.11 Statistical Analysis

Results are reported as arithmetic means and standard deviations, except for residue data which were transformed to common logarithms in order to calculate geometric means and standard deviations. The Statistical Program for Social Sciences (SPSS) was used to perform a one-way analysis of variance followed by Tukey's Alternate Range Test to determine the significance of differences in results among groups. Data were examined by tests for skewness and by inspecting frequency distributions and if necessary transformed to common logarithms prior to statistical analysis. Differences between single groups and controls were determined by t-tests. Unless otherwise indicated, a significance levels of $p < 0.05$ was applied to all statistical tests. Correlations were determined using the Pearson correlation coefficent.

3. CHRONIC DOSING STUDIES

3.1 Objectives

The objectives of this experiment were

1) to examine the effect of Aroclor 1254 on the onset and severity of hepatic porphyrin accumulation and induction of mixed-function oxidases in Japanese quail.

2) to compare the effects of selected PCB congeners on hepatic porphyrin accumulation and induction of polysubstrate monooxygenases in Japanese quail.

A preliminary experiment showed significant enzyme induction but no hepatic porphyria in a strain of Japanese quail after two weeks dosing at 7 mg/kg/day. It was wrongly assumed that the failure to develop porphyria in that preliminary experiment was a function of time of exposure rather than dosage level or the strain of quail used. Therefore, the experiment was designed to examine the time course of porphyrin accumulation rather than a dose response.

The dose used for each congener was a compromise between environmental relevance, based on presence in environmental samples, and efficacy in causing enzyme induction and other sublethal effects in rats. In order to compare the toxicity to birds of certain PCB congeners, and because of cost constraints , I selected a daily dose for # 105 and # 126 approximately equal to the ED50 for AHH induction in Wistar rats (Leece et al., 1985). For congener 153, which does not induce AHH in rats, I selected a dose somewhat higher than for congener 105. The doses used were:

153, 4 mg/kg/day
105, 3 mg/kg/day
126, 0.05 mg/kg/day.

3.2 Results

3.2.1 Liver Porphyrin Levels

3.2.1.1 Aroclor 1254 dosed birds

Porphyrin levels in quail dosed with 7 mg/kg/day of Aroclor 1254 are shown in Fig. 3.1. Chromatograms which compare the porphyrin patterns in dosed versus control quail are in Fig. 3.2. The concentrations in the livers of each bird are shown in Table 3.1. Aroclor 1254 caused a significant increase in HCP levels during the course of the study. Mean HCP levels remained quite constant at 4.5-fold at 4 weeks, with a to 3.1-fold at 8 weeks and 4.4-fold at 12 weeks exposure.

Mean coproporphyrin levels in birds dosed with Aroclor 1254 were similar at 4 and 8 weeks (2.5-fold and 3.1-fold greater than controls respectively) but were 4.7-fold higher after 12 weeks. Variability in the response among individual birds also increased with time of exposure. Protoporphyrin levels also increased after 12 weeks exposure, but the level was not significantly greater than controls.

3.2.1.2 Congener dosed birds

Porphyrin concentrations in livers of birds dosed with PCB congeners are shown in Fig. 3.3. The concentrations in the livers of each bird are shown in Table 3.2. PCB 105 caused a highly significant increase in HCP levels after 2 weeks exposure (4.6-fold). The magnitude of increase over controls was lower after 8 weeks exposure (3.3-fold).

Fig. 3.1 Effects of Aroclor 1254 on hepatic porphyrin levels in female Japanese quail dosed orally with 7 mg/kg/day (see Methods for details). N = 5 for each time point for PCB dosed birds and for control birds except at 12 weeks, where N = 3. The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.

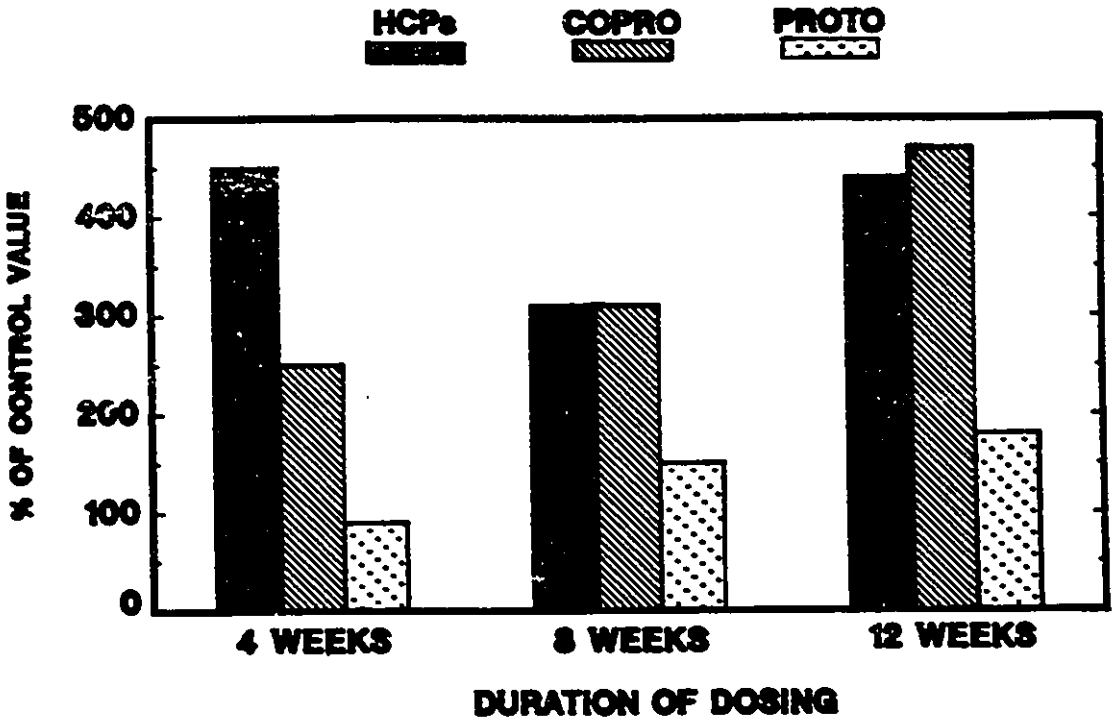
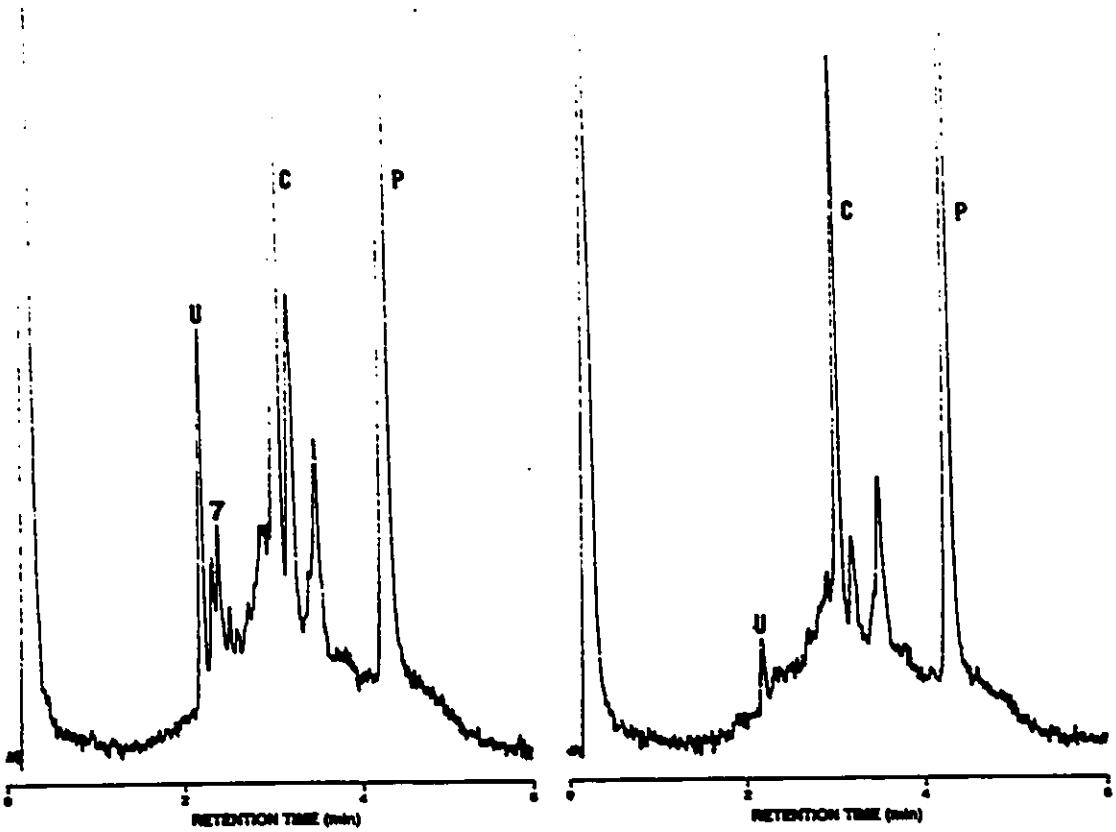

HCPs
COPRO
PROTO
500
400
300
200
100
0
% OF CONTROL VALUE
4 WEEKS
8 WEEKS
12 WEEKS
DURATION OF DOSING

Fig. 3.2 HPLC chromatograms which illustrate the increase in hepatic porphyrin levels in female Japanese quail dosed orally with 7 mg/kg/day of Aroclor 1254 for 12 weeks compared with a bird given only corn oil (See Methods for details) (U = uroporphyrin, C = coproporphyrin, P = protoporphyrin).


U
C
P
RETENTION TIME (min)
AROCLOR 1254
U
C
P
RETENTION TIME (min)
CORN OIL

PCB 126 caused a slight but significant increase in HCP levels after 2 weeks exposure (1.5-fold); after 8 weeks the mean increase relative to controls was greater (3-fold) but the difference was not significant because of increased variability in the response. PCB 153 showed a reversal of this pattern, with no differences evident after 2 weeks, with a slight but statistically significant increase (1.8-fold) in HCPs was measured in this group after 8 weeks exposure.

PCB 105 caused a marked but highly variable increase in accumulation of both coproporphyrin (4.9-fold) and protoporphyrin (2-fold) levels after 2 weeks exposure. After 8 weeks # 105 caused a significant 3-fold increase in coproporphyrin levels, while # 153 caused a significant 2.7 fold increase in coproporphyrin.

3.2.2 PCB residue levels

3.2.2.1 Aroclor 1254 dosed birds

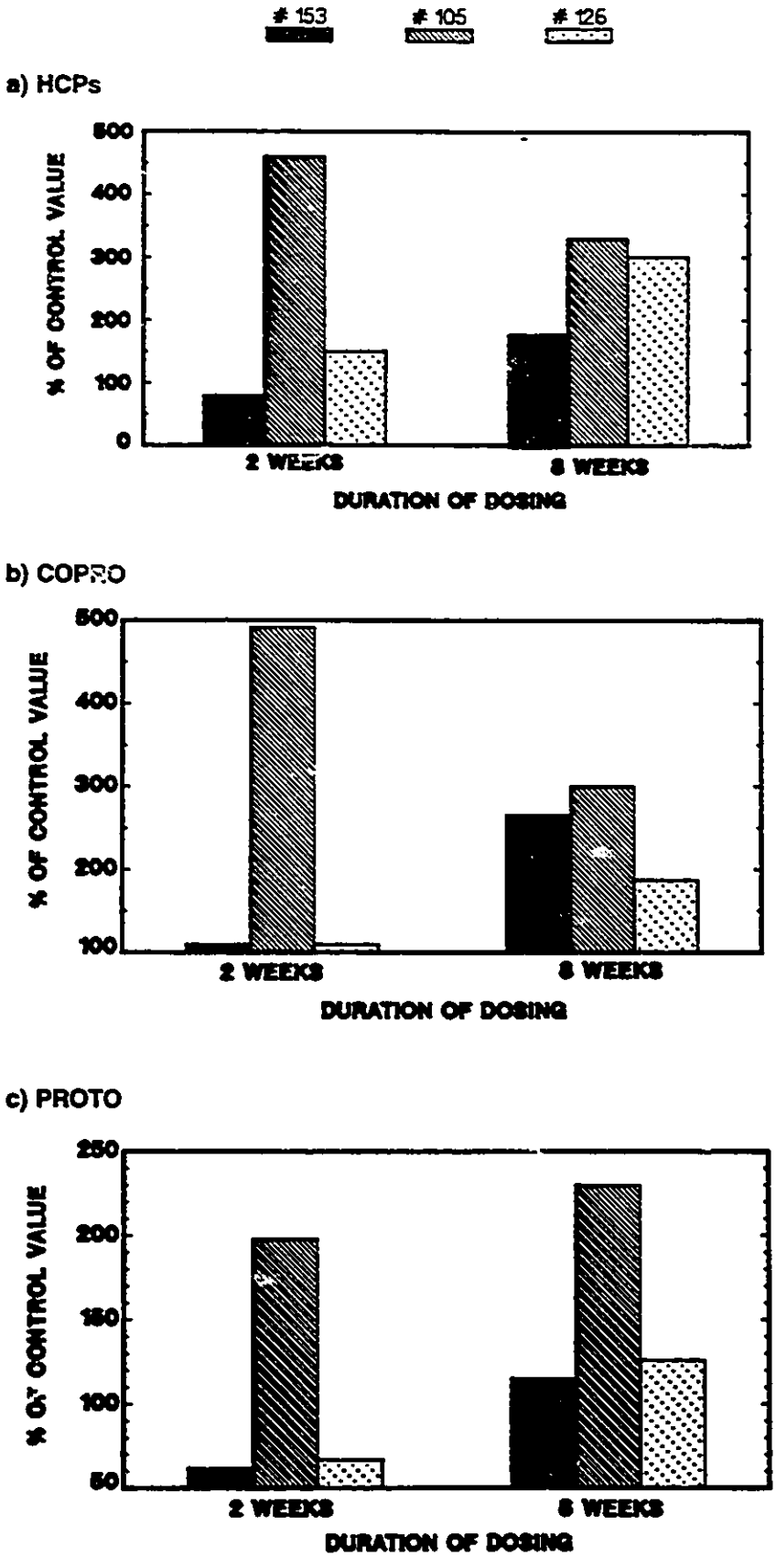
PCB concentrations in livers of quail dosed with 7 mg/kg/day of Aroclor 1254 are shown in Table 3.3. Mean Aroclor 1254 residue levels in liver were the same after 4 and 8 weeks. There was a marked increase after 12 weeks exposure; however, statistical tests were not done as the last two analyses were conducted on pooled samples.

3.2.2.2 Congener dosed birds

Concentrations in livers of birds dosed with PCB congeners are shown in Table 3.4. Liver residue levels of both # 153 and # 105 showed a marked increase after 2 and 8 weeks exposure. PCB # 126 was not detectable after 2 weeks exposure. After 8 weeks, small and highly variable amounts of # 126 were present in the liver.

There was considerable individual variation in residue levels for some compounds. Residue levels of # 105 in birds dosed for two weeks

Fig. 3.3 Effects of PCB congeners on hepatic porphyrin levels in Japanese quail after daily oral dosing (see Methods for details). $N \geq 5$ for each group. The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.


153
105
126
a) HCPs
500
400
300
200
100
0
% OF CONTROL VALUE
2 WEEKS
8 WEEKS
DURATION OF DOSING
b) COPRO
500
400
300
200
100
% OF CONTROL VALUE
2 WEEKS
8 WEEKS
DURATION OF DOSING
c) PROTO
250
200
150
100
50
% OF CONTROL VALUE
2 WEEKS
8 WEEKS
DURATION OF DOSING

varied from 0.2 to 9.9 mg/kg (wet weight); the variation was much less after eight weeks dosing, ranging from 7.6 to 22.0 mg/kg. PCB 153 residue levels in livers of birds dosed for two weeks were much less variable than for birds dosed with # 105, ranging from 9.7 to 22.1 mg/kg.

3.2.3 Liver PSMO activity

3.2.3.1 Aroclor 1254 dosed birds

Hepatic PSMO activity levels in birds dosed with 7 mg/kg/day of Aroclor 1254 are shown in Figs. 3.4 and 3.5. Activity levels for each bird are in Tables 3.5 and 3.6. Aroclor 1254 increased EROD activity over controls by 41-fold after 4 weeks, 82-fold after 8 weeks and 25-fold after 12 weeks. Aroclor 1254 also significantly increased 4-CBP activity over controls by 3-fold at 4 weeks, 4-fold at 8 weeks and 4.5-fold after 12 weeks.

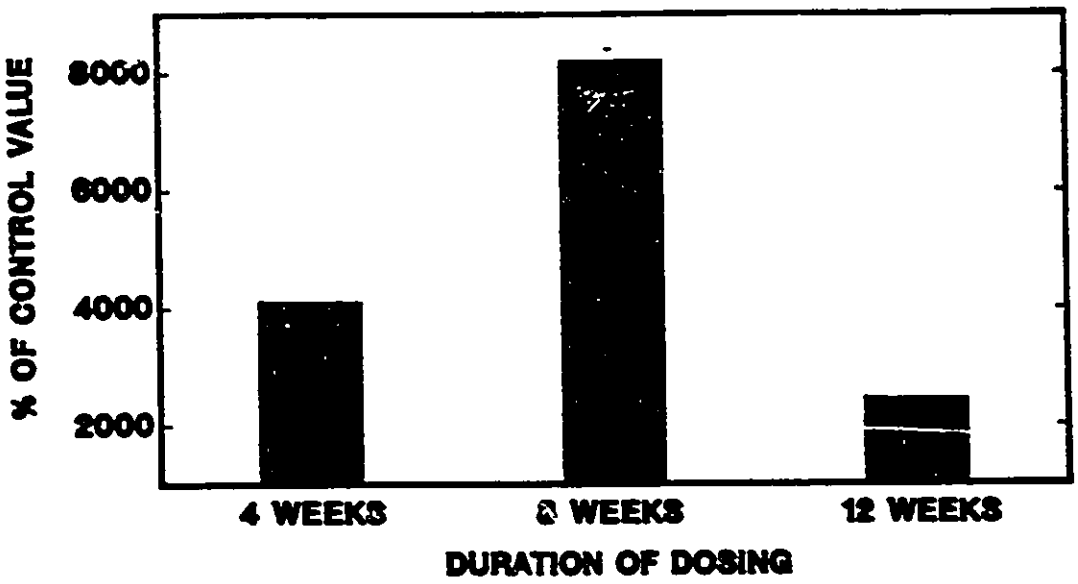
Aroclor 1254 significantly increased APND activity after 4 weeks (3.7-fold), 8 weeks (5.1-fold) and 12 weeks (6.5-fold). Significant increases in AE activity in birds dosed with Aroclor 1254 were evident only after 8 weeks (3.8-fold) and 12 weeks (3.8-fold).

3.2.3.2 Congener dosed birds

Hepatic PSMO activity levels for birds dosed with PCB congeners are shown in Figs. 3.6 and 3.7. Activity levels in each bird are in Tables 3.7 and 3.8. PCB 153 caused no significant change in EROD or 4-CBP levels at 2 weeks, although it did cause a significant 4-fold increase in EROD at 8 weeks. PCB 105 significantly increased EROD levels 27-fold at 2 weeks and 33-fold controls at 8 weeks. PCB 105 also caused highly significant increases in 4-CBP activities, 4.1-fold at 2 weeks and 5.4-fold at 8 weeks. PCB 126 significantly increased EROD activity at both

Fig. 3.4 Effects of Aroclor 1254 on activity of EROD and 4-CBP in liver of Japanese quail dosed orally with 7 mg/kg/day for up to 12 weeks (See Methods for details on dosing of birds). The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.

a) 7-ethoxyresorufin-o-deethylase



b) 4-chlorobiphenyl hydroxylase

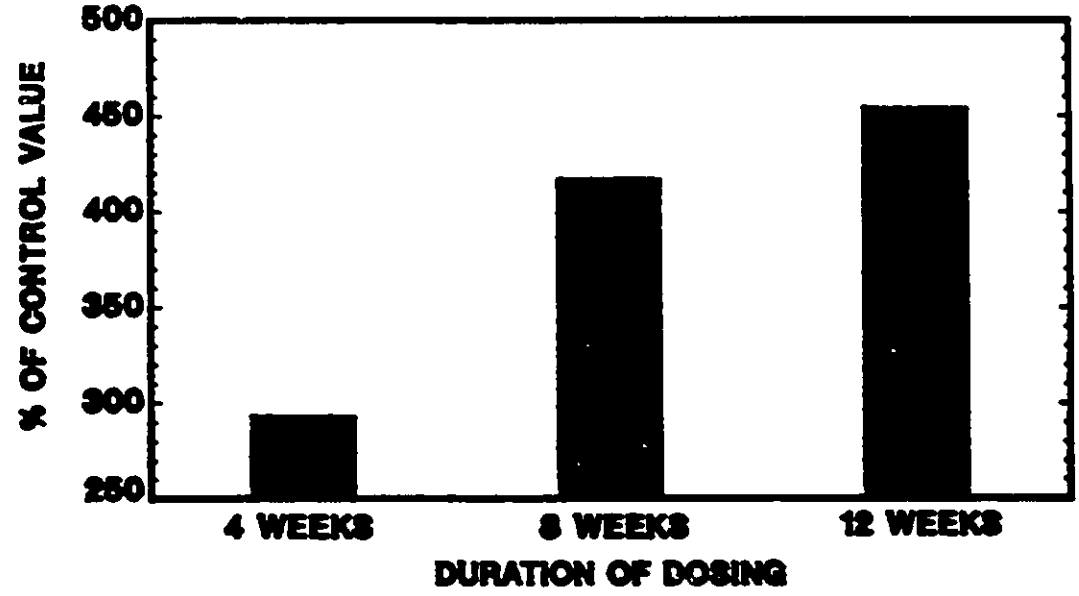
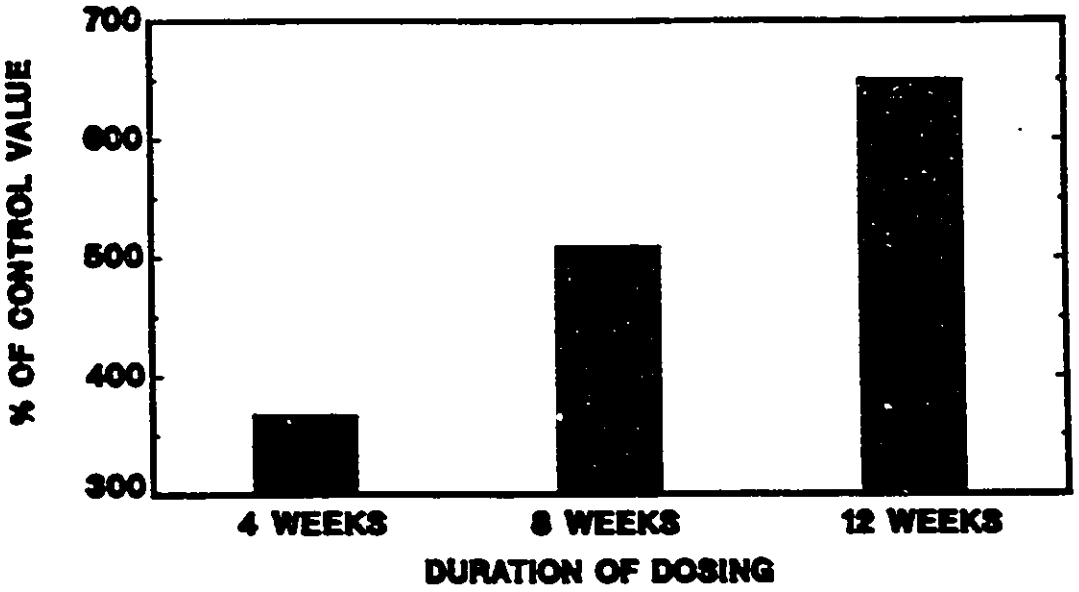
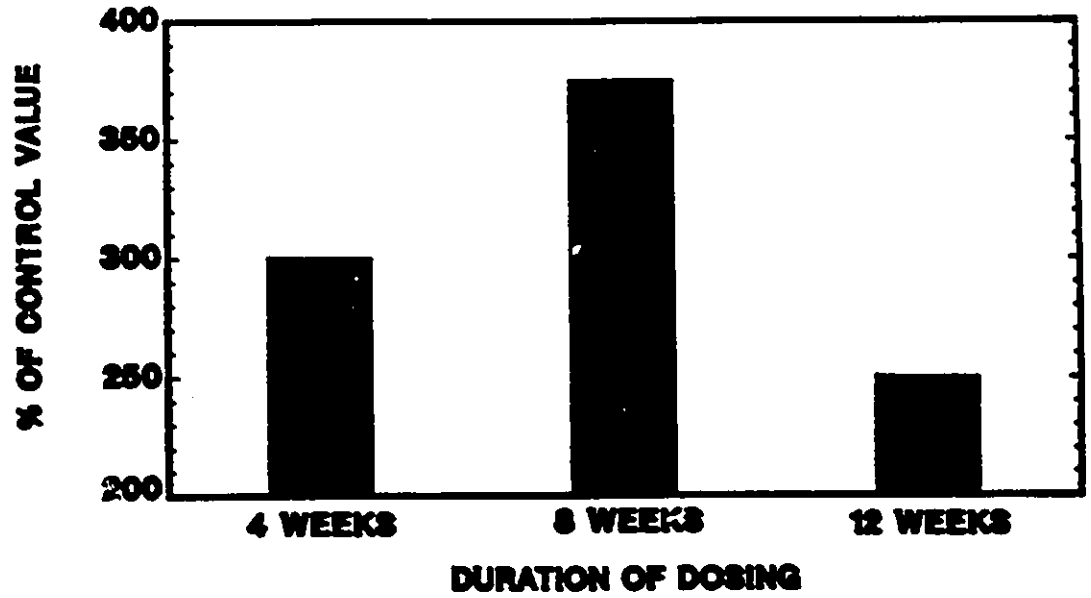


Fig. 3.5 Effects of Aroclor 1254 on activity of AE and APND in liver of Japanese quail dosed orally with 7 mg/kg/day for up to 12 weeks (See Methods for details on dosing of birds). The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.

a) Aminopyrine n-demethylase



b) Aldrin epoxidase



2 weeks (4.5-fold) and at 8 weeks (4-fold), but caused no significant change in 4-CBP activity.

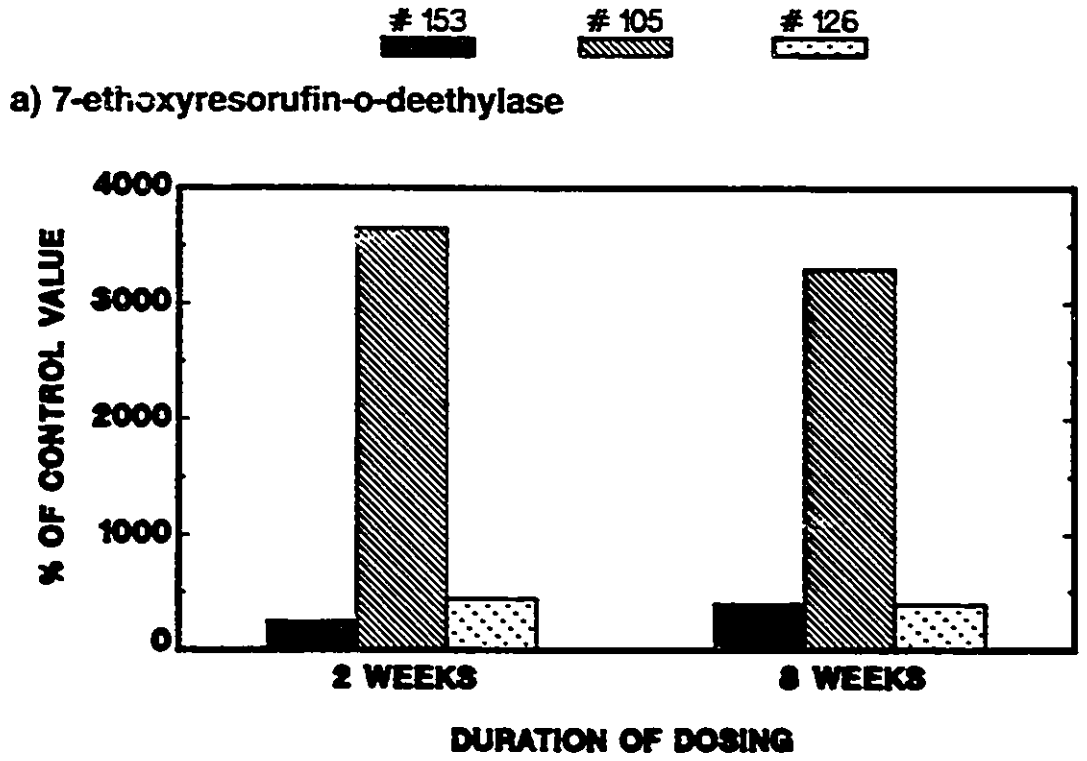
A significant increase in the activity of APND was observed after dosing with PCB 153 for 8 weeks (1.7-fold), but not after 2 weeks dosing. PCB 105 significantly increased APND activity after 2 weeks (3.8-fold) and 8 weeks (4.8-fold). PCB 126 significantly increased APND activity after 2 weeks (2.0-fold) but not after 8 weeks. No significant increase in AE activity occurred as a result of dosing with PCB congeners. PCB 105 caused a 3.6-fold increase in mean AE activity levels over controls after 2 weeks dosing; however, there was considerable variability in the response which was not statistically significant.

3.2.4 Liver and Thymus weights

Liver and thymus weight data for birds dosed with 7 mg/kg/day Aroclor 1254 are shown in Table 3.9 No significant increases in liver weight were measured during the course of exposure to Aroclor 1254 or to any of the individual PCB congeners (Table 3.10). There were no indications of significant change in thymus weight in the birds dosed with Aroclor 1254 .

Among birds dosed with PCB congeners, only the group dosed for 2 weeks with # 126 showed any indication of an effect. Birds from this group had mean thymus weight which was significantly lower ($p < 0.05$, one-tailed t-test) than control birds. However, there was considerable individual variation in thymus weights in many of the groups. Histological examination (by Dr. T. Leighton) of the thymuses showed that birds dosed with # 126 had thinner thymic cortices than control birds, half (3 of 6) of the dosed birds had virtually no thymic cortex. There was , however, some overlap in cortical thickness between two

Fig. 3.6 Effects of PCB congeners on activity of EROD and 4-CBP in liver of Japanese quail dosed orally (See Methods for details on doses). The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.


153
105
126
a) 7-ethoxyresorufin-o-deethylase
4000
3000
2000
1000
0
% OF CONTROL VALUE
2 WEEKS
8 WEEKS
DURATION OF DOSING

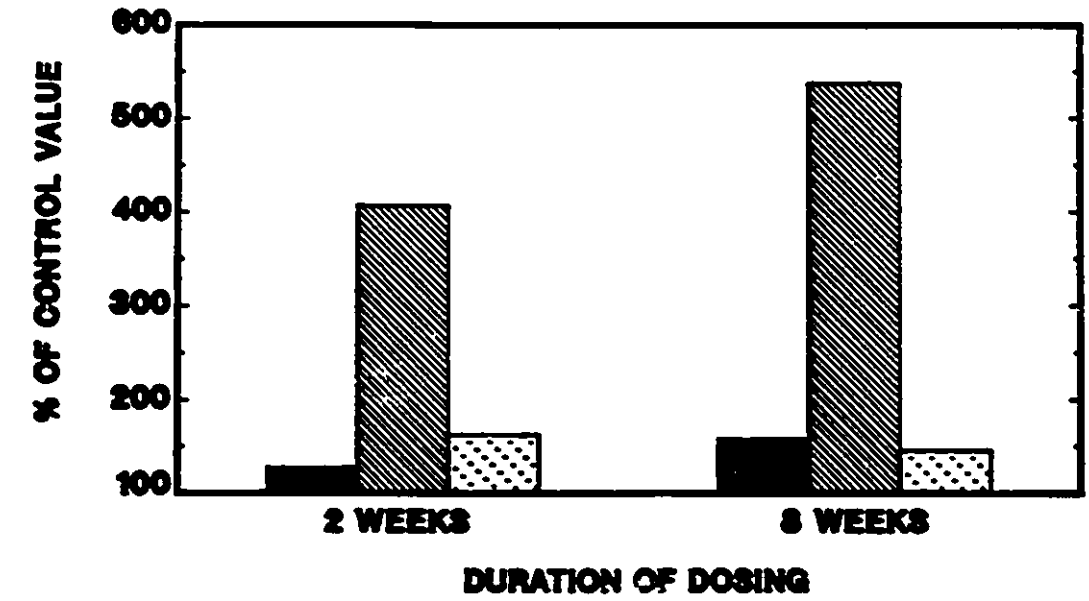
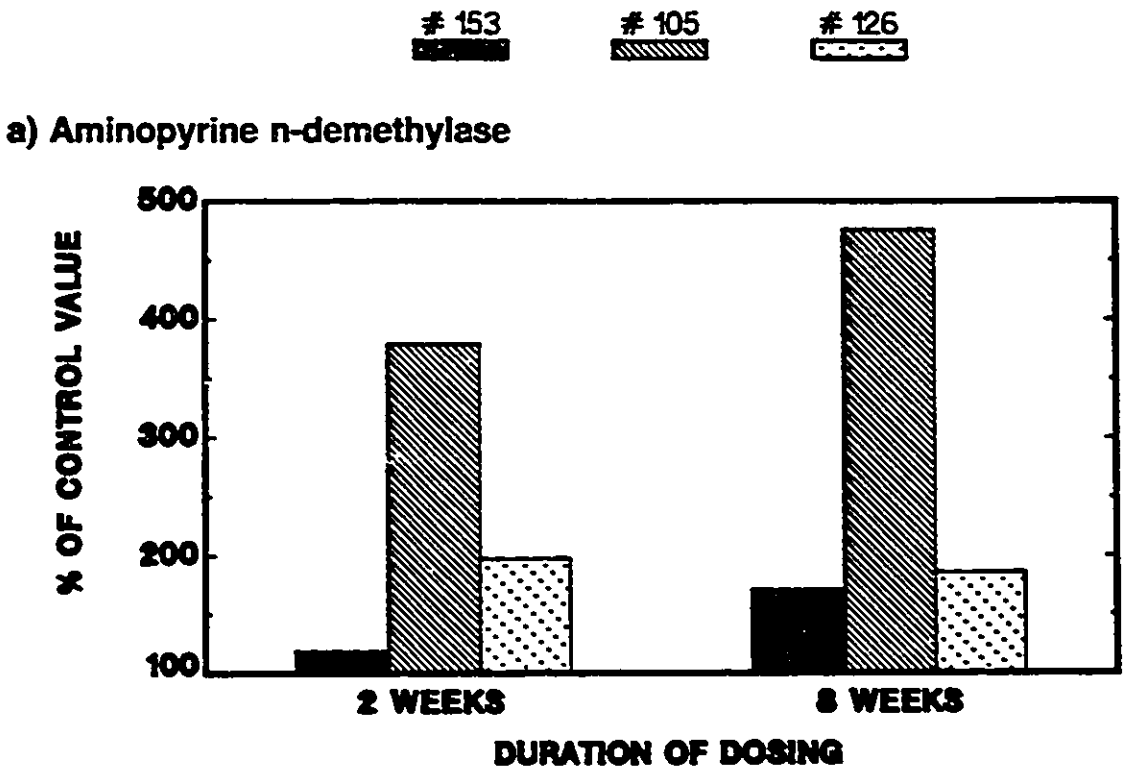
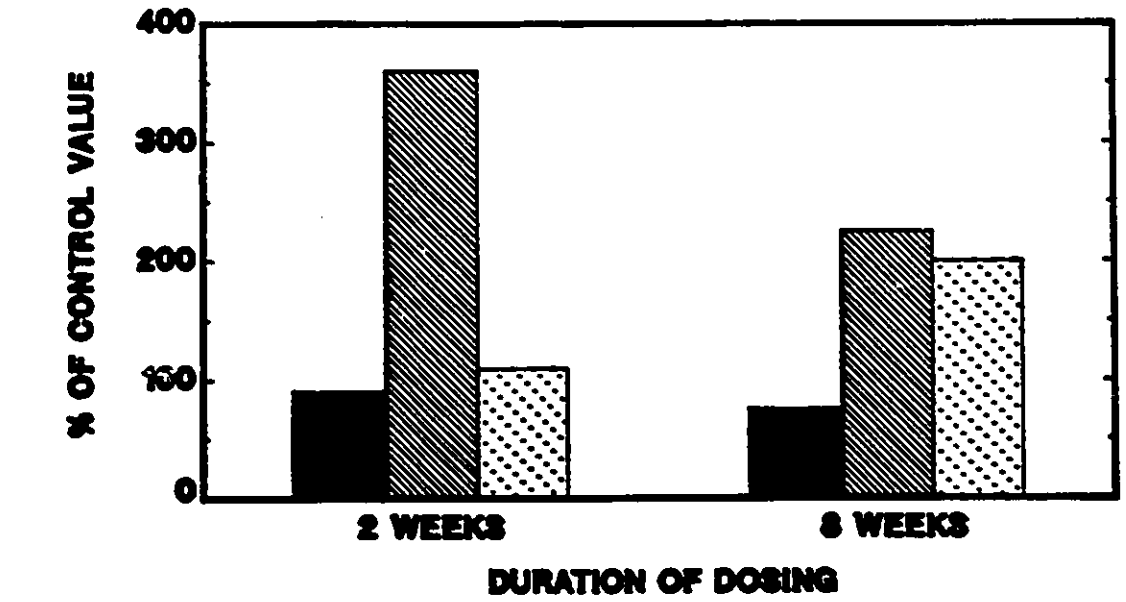

b) 4-Chloro biphenyl hydroxylase
600
500
400
300
200
100
% OF CONTROL VALUE
2 WEEKS
8 WEEKS
DURATION OF DOSING

Fig. 3.7 Effects of PCB congeners on activity of AE APND in liver of Japanese quail dosed orally (See Methods for details on doses). The mean values for each dosed group are plotted as the percentage of the mean value for the control group at that time point.


153
105
126
a) Aminopyrine n-demethylase
% OF CONTROL VALUE
500
400
300
200
100
2 WEEKS
8 WEEKS
DURATION OF DOSING


b) Aldrin epoxidase
% OF CONTROL VALUE
400
300
200
100
0
2 WEEKS
8 WEEKS
DURATION OF DOSING

individual birds dosed with # 126 and individual control birds. Depletion of lymphocytes from the thymic cortex was also marked in the # 126 dosed birds.

3.2.5 Health of Birds

Five quail died during the course of the study, two dosed and three control birds. All other birds appeared healthy, ate regularly and gained weight throughout the study. The condition of the birds which died and the possible causes of death were as follows:

One bird in the 8 week control group died of no obvious cause, the bird being healthy in appearance and normal in weight. One bird in the 12 week control group became egg-bound and died of cloacal haemorrhage 1 week into the study. A second bird in the 12 week control group broke its leg and died at 8 weeks apparently of a resulting infection.

One bird in the 8 week group dosed with # 153 died after 4 weeks dosing, it was normal in appearance and weight. One bird in the # 105 eight week group died after five and half weeks dosing. It weighed 91 grams less than the mean weight of other birds in its group at that point, having lost 20 grams since the onset of dosing, while the rest of the group averaged a 42 gram weight gain. Prior to death the bird was weak and had stopped feeding. Its muscles were atrophied. Thymus weight was 0.0252, much less than mean control weight. APND activity was markedly elevated at 26.6 nmol/min/mg. Liver porphyrin levels, AE, EROD and 4-CBP activity levels were also elevated but were within the range of the rest of the group.

3.3 Discussion

3.3.1 Effects of Aroclor 1254

The results of this experiment show that Aroclor 1254 causes elevation of liver porphyrin levels at a dose of 7 mg/kg/day over a 4 to 12 week exposure period. PCB mixtures have been shown to cause porphyria in a variety of animal species and in isolated cultures of animal cells (Marks, 1985). Aroclor 1254 caused significant accumulation of total porphyrins in rats fed 7.9 mg/kg/day (Goldstein *et al.*, 1974). In that study, as in many other investigations of porphyria in mammals, total porphyrin accumulation was not detected until some weeks or months after exposure. However, Kennedy *et al.* (1986) have shown that in rats dosed with HCB, the highly-carboxylated porphyrins are significantly higher than controls within 4 days of dosing. Miranda *et al.* (1986) reported that in Japanese quail, total porphyrins reach maximal elevation in liver within 48 hours after a single high dose of Aroclor 1254 . In our preliminary experiments, one strain of Japanese quail (Archambault supplier) dosed for 2 weeks with 7 mg/kg/day Aroclor 1254 failed to develop porphyria, while a second strain (Macdonald college supplier) dosed at the same level for 4, 8 and 12 weeks showed significant accumulation of both HCPs and coproporphyrin (Fig. 3.1, Table 3.1). While the difference was initially ascribed to length of exposure, it may have been due to genetic differences between the strains of quail. Bursian *et al.* (1983) reported that different genetic lines of Japanese quail varied in their biochemical response to polybrominated biphenyl (PBB) exposure. Subsequent experiments (Chapter 4) also showed massive porphyrin accumulation in the Macdonald strain within five days of receiving a single high dose of Aroclor 1254.

3.3.2 Comparative effects of PCB congeners

As described in the methods, the quail in this study were given a dose of PCBs # 105 and # 126 which was based on the ED50 value for *in vivo* AHH induction in Wistar rats (Leece *et al.*, 1985). Both # 105 and # 126 caused HCP accumulation, although # 105 caused a greater degree of HCP accumulation as well as significant COPRO and PROTO accumulation at both time points. PCB # 105 also increased P-450-MC type activity as measured by both EROD and 4-CBP; it also increased the P-450-PB type activity as measured by APND, but not by AE. In contrast # 126 caused only slight increases in EROD activity and no effect on 4-CBP; it did however increase APND activity significantly after two weeks. Therefore, the relative potency of the different congeners to induce P-450-MC type induction was different in quail than in rats.

Table 3.11 Estimation of TCDD toxic equivalents of PCB congener doses

PCB	dose		TEF*		TCDD equivalents	Reference
126	0.05 mg/kg	*	0.40	=	0.02 mg/kg	1
105	3.0 mg/kg	*	0.0011	=	0.0033 mg/kg	1
Aroclor 1254	7.0 mg/kg	*	0.00001	=	0.0001 mg/kg	2

* TEF - toxic equivalence factor, 1 - Sawyer & Safe, 1982;
2 - Sawyer *et al.*, 1983.

The toxicity of PHAH type compounds can also be estimated by comparing their toxicity to that of 2378-TCDD based on its *in vitro* potency to induce AHH and EROD in rat hepatoma H-4-II E cells, which has

been correlated with rat in vivo measurements (Sawyer et al., 1984; Leece et al., 1985; Ontario Ministry of Environment, 1985). Using the method of Sawyer et al. (1984), "TCDD toxic equivalents" were determined for doses used in this experiment (Table 3.11). Based on this analysis, # 126 should have been much more effective than # 105 or Aroclor 1254, if the relative potencies derived from the above cited rat studies were applicable to birds.

PCB 153 had no effect on porphyrin levels after two weeks exposure; however, after eight weeks, HCPs were slightly elevated and COPRO levels were comparable to levels in birds dosed with either Aroclor 1254 or PCB 105 for 8 weeks. The slight but significant increase in HCP levels caused by # 153 is not consistent with structure activity analyses of PCB congeners in cell culture which predicts that di-ortho substituted (2,2'-) PCBs do not cause porphyria (Sassa et al., 1986). Although Sano et al. (1985) reported that two of the di-ortho PCBs (#s 128, 155) tested were at least mildly porphyrinogenic in chick embryo liver cells. Rifkind et al. (1984) reported that at higher doses # 153 injected into chicken eggs invoked a P-448 type response and caused liver damage, which they attributed to proven contamination of their PCB 153 batch by 1.5 ppm of TCDF. However, Goldstein et al. (1976) reporting that 400 ppm of # 153 in the diet of chicks caused gross accumulation of hepatic porphyrins also showed that up to 5 ug/kg of 2378-TCDF did not cause porphyrin accumulation in chicks, and therefore excluded this explanation for effects caused by PCB 153. As discussed in more detail in Chapter 4, the PCB 153 used in the experiments described in these two chapters was found to contain various dioxin and furan impurities, in particular 1.25 ppm of 2378-TCDD. Therefore, birds dosed

with 153 would also have received about 5 ng/kg/day of TCDD, which may account for the minor EROD induction and HCP accumulation observed after 8 weeks dosing with # 153. A 6 nmol/egg dose of TCDD caused maximum EROD induction in chick embryos (Rifkind et al., 1985).

3.3.3 Relationship between residue levels and biochemical parameters

Differences in PCB liver concentrations cannot account for the differences observed between chemicals and/or time points in either porphyrin accumulation or PSMO induction. Also, there were no correlations between liver residue levels for Aroclor 1254 or any of the PCB congeners in individual birds and the hepatic functions measured. For example although Aroclor 1254 liver residue levels increased markedly at 12 weeks exposure compared to 4 or 8 weeks, only COPRO increased simultaneously while other parameters were stable or decreased. While mean residue levels of # 105 also increased from 2.6 mg/kg after 2 weeks to 12.2 mg/kg after eight weeks, porphyrin levels and PSMO activity actually decreased. For # 126, as residue levels went from non-detectable after two weeks to 0.1 mg/kg after eight weeks, there was no pattern of increasing effect on hepatic functions. Only for # 153 was there an increase in residue levels associated with slight increases in the effect of this compound on both porphyrin levels and activity of EROD and APND. Concerning the relationship between congener accumulation and effects, Goldstein et al. (1976) for chick liver and fat and Bunyan & Page (1978) in Japanese quail heart both reported among the PCBs tested that PCB 169 (3,3',4,4',5,5'-HCB) was both most accumulative and caused the most dramatic effects including lethality.

There were striking differences in residue levels among individual birds, especially those dosed with # 105 for two weeks. This variance may be the result of individual variation in metabolism, although in the rat Yamamoto et al. (1976) did not detect any metabolites of # 105 in rat urine up to eight days after dosing. Variation in intestinal absorption is also possible as Yamamoto et al. (1976) also reported that an average of 32.3 % of a 150 mg/kg dose of # 105 administered orally to rats in soybean bean oil was excreted unchanged in feces within one day of dosing.

3.3.4 Induction of polysubstrate monooxygenases

Aroclor 1254 increased both EROD and 4-CBP, the enzymes used to assay for P-450-MC type activity and also APND and AE which were used for P-450-PB activity. PCB mixtures have been characterized as mixed inducers in studies with a variety of animal species (Safe, 1985). Increased activity of both EROD and 4-CBP and APND and AE in birds dosed with # 105 is consistent with this congener having been characterized as a mixed inducer in rats (Parkinson et al., 1980). Significant APND induction by # 126 was not expected. Leece et al., reported no induction of APND by # 126 in rats. However, APND induction by PCB 77 has been reported for chickens (Jones et al., 1985). Differences between birds and mammals in their response to the classical inducers, such as phenobarbitol and methylcholanthrene have been reported in other studies and are discussed in more detail in Chapter 4.

The activity of 4-CBP was increased significantly only by Aroclor 1254 and PCB 105. Parkinson et al. (1980) reported that mixed inducers caused the greatest increase in 4-CBP activity in rats; they also reported that 4'-chloro-4-biphenylol was consistently the primary

metabolite (87-93 %). In quail, we observed a second unknown peak which varied according to the chemical pretreatment. This may indicate a basic difference between rats and quail in the ratio of metabolites produced and should be further investigated. The degree of 4-CBP induction in quail was also considerably less than in rats. Parkinson et al. reported that 150 umol/kg of PCB #189 (2,3,3',4,4',5,5'-HpCB) increased 4-CBP activity by 25-fold. In quail maximal induction was in the order of 5-fold.

There was considerable within group variation in aldrin epoxidase activity. Statistically significant induction occurred only in birds dosed with Aroclor 1254 for 8 and 12 weeks. Rinzky & Perry (1983) also reported considerable variation in AE results in both untreated chickens and chickens dosed with Aroclor 1254.

3.3.5 Relationship between porphyrin accumulation and PSMO activity

One of the objectives of this study was an examination of the possible relationship between porphyria and PSMO induction. Recent studies using specific inhibitors of PSMO activity have shown that P-450-MC type induction is necessary prior to development of porphyria in vitro in chick embryo hepatocyte cells (Debets et al., 1980; Sinclair et al, 1986; 1987). HCP accumulation in quail was significant only in dosage group where there was also significant EROD induction. However, significant HCP accumulation also only occurred in groups with significant APND induction. In order to normalize the data, the log10 HCP concentration for all dosed and control birds was plotted against the non-transformed results for the PSMO assays. There were highly significant correlations for APND ($r = 0.7097$), 4-CBP ($r = 0.6716$) and

EROD (r = 0.5140); however, these correlations do not necessarily indicate a cause effect relationship.

3.3.6 Liver and thymus weight

No other signs of toxicity such as liver weight increase or atrophy of the thymus were observed in quail which showed elevated prophyrin levels and induction of MFOs. In other studies, chickens fed 400 ppm of Aroclor 1260 for 60 days had significantly enlarged livers and porphyria (Vos & Koeman, 1970). However, quail fed up to 100 ppm of Aroclor 1260 for seven days did not show significant increases in liver weight per unit body weight, although most had fluorescent livers at 100 mg/kg (Vos et al., 1971).

The significant decrease in thymus weight and histological indications of lymphocyte depletion in quail fed PCB 126 for two weeks may have been a spurious result, as there was so much variability in the thymus weights among groups. In addition, thymus weights in birds fed # 126 for 8 weeks were greater than controls. There was no correlation with other parameters as # 126 caused only minimal EROD and 4-CBP induction. Leece et al. (1985) reported that in Wistar rats, the ED50 for thymic atrophy was only 0.9-fold the EROD ED50 while for #105 the ED50 for thymic atrophy was 16-fold that for EROD.

Nevertheless, the possibility that PCB 126 caused thymic atrophy at a low dose should probably be further examined. Reduction of immunocompetance in ducklings caused by PCBs, which is accompanied by no other observed effects, has been known for some time (Friend & Trainer, 1970).

3.4 Conclusions

The results show that chronic exposure to moderate levels of Aroclor 1254 caused persistent porphryia and induction of EROD, 4-CBP and APND in quail. The results support the potential of these assays for use as biochemical measures of avian exposure to PCBs.

The results suggest that the relative toxicity of PCB congeners in birds may be quite different from rats. Further studies should be undertaken to assess the applicability of data on mammals to understanding PHAH toxicity to birds. This would aid investigations, such as those of Kubiak *et al.* (1988), who used TCCD toxic equivalence factors determined from rat studies to assess the contribution of PCB congeners and other PHAHs to reproductive effects in a wild bird population.

TABLE 3.1 Hepatic porphyrin levels in Japanese Quail dosed orally with Aroclor 1254 at a rate of 7 mg/kg day. Experimental groups which are significantly different from controls are followed by the significance level.

Duration of dosing and treatment	Quail	HCPs (pmol/g)	coproporphyrin (pmol/g)	protoporphyrin (pmol/g)
4 weeks				
Corn oil	1	10	45	108
	2	16	220	198
	3	13	104	325
	4	4	27	42
	5	5	49	131
	Mean ± S.D.	10 ± 5	89 ± 79	161 ± 107
Aroclor 1254	1	53	159	90
	2	33	174	157
	3	37	285	199
	4	65	192	121
	5	39	279	157
	Mean ± S.D.	45^{c} ± 13	218^{a} ± 60	145 ± 41
8 weeks				
Corn oil	1	7	81	47
	2	14	93	60
	3	7	78	111
	4	4	53	130
	5	6	82	108
	Mean ± S.D.	8 ± 4	77 ± 15	91 ± 36
Aroclor 1254	1	38	235	123
	2	31	257	157
	3	25	330	120
	4	21	260	156
	5	9	128	111
	Mean ± S.D.	25^{b} ± 11	242^{c} ± 73	134 ± 21
12 weeks				
Corn oil	1	10	132	143
	2	7	66	160
	3	7	88	135
	Mean ± S.D.	8 ± 2	95 ± 34	146 ± 13
Aroclor 1254	1	37	506	219
	2	40	438	341
	3	23	675	362
	4	14	325	213
	5	60	288	159
	Mean ± S.D.	35^{a} ± 18	446^{b} ± 155	259 ± 88

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.2 Hepatic porphyrin levels in Japanese Quail dosed orally with PCB congeners. Experimental groups which are significantly different from controls are followed by the significance level.

Duration of dosing, treatment, and dose	Quail	HCPs (pmol/g)	Copro-porphyrin (pmol/g)	Proto-porphyrin (pmol/g)
2 weeks				
Corn oil	1	10	45	108
	2	16	220	198
	3	13	104	325
	4	4	27	42
	5	5	49	131
	Mean ± S.D.	10 ± 5	89 ± 79	161 ± 107
# 153, 4 mg/kg/day	1	11	135	135
	2	9	111	121
	3	9	80	64
	4	8	85	111
	5	5	64	37
	6	6	80	132
	Mean ± S.D.	8 ± 2	93 ± 26	100 ± 40
105, 3 mg/kg/day	1	17	177	121
	2	50	221	94
	3	47	372	814
	4	23	210	220
	5	70	417	309
	6	69	1231	350
	Mean ± S.D.	46[c] ± 22	438 ± 400	318 ± 263
126, 0.05 mg/kg/day	1	10	41	73
	2	33	115	103
	3	19	109	143
	4	13	79	100
	5	11	147	93
	6	9	104	132
	Mean ± S.D.	15[a] ± 6	99 ± 36	107 ± 26
8 weeks				
Corn oil	1	7	81	47
	2	14	93	60
	3	7	78	111
	4	4	53	130
	5	6	82	108
	Mean ± S.D.	8 ± 4	77 ± 15	91 ± 36
153, 4 mg/kg/day	1	11	297	99
	2	14	147	92
	3	15	117	107
	4	10	104	90
	5	18	354	139
	Mean ± S.D.	14[a] ± 3	204[a] ± 114	105 ± 20

Table 3.2, contin.

105, 3 mg/kg/day	1	53	440	198
	2	28	214	345
	3	10	156	211
	4	12	114	83
	Mean ± S.D.	26[a] ± 20	231[b] ± 145	209 ± 107
126, 0.05 mg/kg/day	1	43	126	117
	2	22	53	97
	3	48	306	99
	4	9	175	128
	5	9	130	103
	6	11	73	143
	Mean ± S.D.	24 ± 18	144 ± 90	115 ± 18

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.3 PCB residue levels (mg/kg, wet weight) in liver of Japanese quail dosed orally with Aroclor 1254 at a rate of 7 mg/kgday.

	Duration of dosing and treatment					
	4 weeks		8 weeks		12 weeks	
	corn oil	Aroclor 1254	corn oil	Aroclor 1254	corn oil	Aroclor 1254
Mean	ND	9.58	ND	9.90*	ND	29.8*
± S.D.		± 6.69				
N	5	5	5	5	3	5

ND - non-detectable
* - pooled sample

TABLE 3.4 PCB congener residue levels (mg/kg, wet weight) in liver of Japanese quail dosed orally with PCB congeners.

	Duration of dosing, treatment, (dose, mg/kg/day)							
	2 weeks				8 weeks			
Quail	Corn oil	153 (4)	105 (3)	126 (0.05)	Corn oil	153 (4)	105 (3)	126 (0.05)
1	ND	17.5	2.54	ND	ND	52.6*	10.7	0.006
2	ND	19.9	0.28	ND	ND	52.6	7.6	0.018
3	ND	22.1	-	ND	ND	52.6	22.0	0.002
4	ND	12.3	0.27	ND	ND	52.6	8.4	0.490
5	ND	9.7	9.92	ND	ND	52.6		0.006
6		12.8	0.21	ND				0.026
Mean	ND	15.7	2.6	ND	ND	52.6	12.2	0.091
± S.D.		4.9	4.2				6.7	0.196
N	5	6	6	6	5	5	4	6

* - pooled sample
(-) not analysed
ND - non-detectable

TABLE 3.5 Microsomal total protein and monooxygenase activities in liver of Japanese Quail dosed orally with aroclor 1254 at a rate of 7 mg/kg day. Experimental groups which are significantly different (t-test) from controls are followed by the significance level.

Duration of dosing and treatment	Quail	Total Protein (ug/ml)	7-ethoxyresorufin O-deethylase (nmols/mg/min)	4-chlorobiphenyl hydroxylase (nmols/mg/min)
4 weeks				
Corn oil	1	2.6	0.02	0.27
	2	3.2	0.01	0.54
	3	2.2	0.04	0.55
	4	1.5	0.03	0.21
	5	3.3	0.02	0.49
	Mean ± S.D.	2.6 ± 0.7	0.02 ± 0.02	0.41 ± 0.16
Aroclor 1254	1	2.3	1.19	1.01
	2	2.3	0.34	0.91
	3	4.0	0.61	0.84
	4	3.1	0.81	1.51
	5	2.6	1.35	1.71
	Mean ± S.D.	2.9 ± 0.7	0.82^{b} ± 0.38	1.20^{a} ± 0.40
8 weeks				
Corn oil	1	2.9	0.01	0.12
	2	3.8	0.01	0.40
	3	4.6	0.01	0.13
	4	3.1	0.02	0.18
	5	3.4	0.01	0.38
	Mean ± S.D.	3.6 ± 0.7	0.01 ± 0.00	0.24 ± 0.14
Aroclor 1254	1	4.6	0.63	1.24
	2	3.7	0.66	0.90
	3	5.2	1.73	1.42
	4	4.9	0.52	0.70
	5	4.5	0.56	0.77
	Mean ± S.D.	4.6 ± 0.6	0.82^{c} ± 0.51	1.00^{c} ± 0.40
12 weeks				
Corn oil	1	4.7	0.01	0.13
	2	2.8	0.00	0.29
	3	3.7	0.05	0.30
	Mean ± S.D.	3.7 ± 1.0	0.02 ± 0.02	0.24 ± 0.14
Aroclor 1254	1	4.8	0.55	0.87
	2	3.4	0.26	1.22
	3	5.7	0.48	1.17
	4	3.8	0.33	0.89
	5	5.2	0.85	1.30
	Mean ± S.D.	4.5 ± 1.0	0.49^{b} ± 0.23	1.09^{c} ± 0.20

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.6 Microsomal monooxygenase activities in liver of Japanese quail dosed orally with aroclor 1254 at a rate of 7 mg/kg day. Experimental groups which are significantly different (t-test) from controls are followed by the significance level.

Duration of dosing and treatment	Quail	Aminopyrine N-demethylase (nmols/mg/min)	Aldrin epoxidase (nmols/mg/min)
4 weeks			
Corn oil	1	3.2	0.03
	2	5.9	0.24
	3	5.5	0.04
	4	2.6	0.12
	5	3.3	0.08
	Mean ± S.D.	4.2 ± 1.6	0.10 ± 0.09
Aroclor 1254	1	12.0	0.29
	2	12.0	0.02
	3	13.1	0.02
	4	16.2	0.15
	5	23.9	1.04
	Mean ± S.D.	15.4[c] ± 5.0	0.30 ± 0.43
8 weeks			
Corn oil	1	2.0	0.02
	2	2.6	0.06
	3	1.4	0.02
	4	1.3	0.02
	5	3.2	0.07
	Mean ± S.D.	2.1 ± 0.8	0.04 ± 0.02
Aroclor 1254	1	13.8	0.14
	2	8.7	0.05
	3	17.2	0.31
	4	6.4	0.07
	5	7.3	0.17
	Mean ± S.D.	10.7[c] ± 4.6	0.15[a] ± 0.10
12 weeks			
Corn Oil	1	0.9	0.02
	2	2.0	0.04
	3	2.6	0.06
	Mean ± S.D.	1.8 ± 0.9	0.04 ± 0.02
Aroclor 1254	1	10.8	0.07
	2	11.1	0.14
	3	10.4	0.08
	4	9.3	0.17
	5	16.9	0.08
	Mean ± S.D.	11.7[c] ± 3.0	0.11[a] ± 0.04

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.7 Microsomal total protein and monooxygenase activities in liver of Japanese Quail dosed orally with PCB congeners. Experimental groups which are significantly different (t-test) from controls are followed by the significance level.

Duration of dosing, treatment and dose	Quail	Total Protein (ug/ml)	7-ethoxyresorufin O-deethylase (nmols/mg/min)	4-chlorobiphenyl hydroxylase (nmols/mg/min)
2 weeks				
Corn oil	1	2.6	0.02	0.27
	2	3.2	0.01	0.54
	3	2.2	0.05	0.55
	4	1.5	0.03	0.21
	5	3.3	0.02	0.49
	Mean ± S.D.	2.6 ± 0.7	0.02 ± 0.02	0.41 ± 0.16
153, 4 mg/kg/day	1	2.4	0.03	0.85
	2	2.3	0.05	0.47
	3	2.1	0.01	0.48
	4	3.2	0.05	0.29
	5	3.0	0.04	0.32
	6	1.8	0.11	0.68
	Mean ± S.D.	2.5 ± 0.5	0.05 ± 0.03	0.52 ± 0.22
105, 3 mg/kg/day	1	1.9	1.77	2.73
	2	3.4	0.88	1.61
	3	4.2	0.57	1.60
	4	2.4	0.09	1.42
	5	3.8	0.15	1.26
	6	2.7	0.92	1.37
	Mean ± S.D.	3.1 ± 0.9	0.73[a] ± 0.62	1.67[c] ± 0.54
126, 0.05 mg/kg/day	1	2.4	0.02	0.74
	2	2.1	0.13	0.92
	3	3.7	0.04	0.34
	4	2.1	0.04	0.97
	5	3.9	0.10	0.57
	6	3.1	0.34	0.46
	Mean ± S.D.	2.9 ± 0.8	0.09[a] ± 0.09	0.67 ± 0.25
8 weeks				
Corn oil	1	2.9	0.01	0.12
	2	3.8	0.01	0.40
	3	4.6	0.01	0.13
	4	3.1	0.01	0.18
	5	3.4	0.01	0.38
	Mean ± S.D.	3.6 ± 0.7	0.01 ± 0.00	0.24 ± 0.14
153, 4 mg/kg/day	1	5.9	0.04	0.40
	2	3.7	0.04	0.55
	3	3.6	0.01	0.27
	4	4.2	0.10	0.30
	5	5.1	0.02	0.39
	Mean ± S.D.	4.5 ± 1.0	0.04[b] ± 0.03	0.38 ± 0.11

Table 3.7, contin.

105, 3 mg/kg/day	1	5.4	0.51	1.19
	2	4.4	0.30	1.14
	3	3.2	0.17	1.00
	4	5.2	0.35	0.81
Mean ± S.D.		4.5 ± 1.0	0.33[c] ± 0.14	1.29[c] ± 0.43
126, 0.05 mg/kg/day	1	2.2	0.05	0.52
	2	3.9	0.03	0.29
	3	5.1	0.01	0.19
	4	4.2	0.05	0.45
	5	4.0	0.08	0.32
Mean ± S.D.		3.9 ± 1.1	0.04[b] ± 0.03	0.35 ± 0.13

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.8 Microsomal monooxygenase activities in liver of Japanese quail dosed orally with individual PCB congeners. Experimental groups which are significantly different from controls are followed by the significance level.

Duration of dosing, treatment and dose	Quail	Aminopyrine N-demethylase (nmols/mg/min)	Aldrin epoxidase (nmols/mg/min)
2 weeks			
Corn oil	1	3.2	0.03
	2	5.9	0.24
	3	5.5	0.04
	4	2.6	0.12
	5	3.3	0.08
	Mean ± S.D.	4.2 ± 1.6	0.10 ± 0.09
153, 4 mg/kg/day	1	4.4	0.11
	2	4.0	0.10
	3	2.9	0.07
	4	4.8	0.13
	5	4.0	0.05
	6	9.8	0.09
	Mean ± S.D.	5.0 ± 2.4	0.09 ± 0.03
105, 3 mg/kg/day	1	24.8	0.03
	2	20.9	1.05
	3	14.5	0.22
	4	6.7	0.03
	5	9.2	0.08
		19.1	0.47
	Mean ± S.D.	15.9[b] ± 7.1	0.36 ± 0.37
126, 0.05 mg/kg/day	1	5.0	0.09
	2	15.1	0.26
	3	5.6	0.07
	4	7.5	0.10
	5	8.6	0.09
	6	7.8	0.03
	Mean ± S.D.	8.3[a] ± 3.6	0.11 ± 0.08
8 weeks			
Corn oil	1	2.0	0.02
	2	2.6	0.06
	3	1.4	0.02
	4	1.3	0.02
	5	3.2	0.07
	Mean ± S.D.	2.1 ± 0.8	0.04 ± 0.02
153, 4 mg/kg/day	1	3.1	0.04
	2	4.4	0.03
	3	2.0	0.02
	4	4.7	0.04
	5	3.9	0.02
	Mean ± S.D.	3.6[a] ± 1.1	0.03 ± 0.01

Table 3.8, contin.

105, 3 mg/kg/day	1	12.3	0.04
	2	10.7	0.12
	3	9.3	0.15
	4	7.6	0.05
	Mean ± S.D.	10.0[c] ± 2.0	0.09 ± 0.05
126, 0.05 mg/kg/day	1	5.6	0.07
	2	3.6	0.06
	3	1.4	0.01
	4	4.9	0.04
	5	3.8	0.20
	Mean ± S.D.	3.9 ± 1.0	0.08 ± 0.07

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 3.9. Liver and thymus weights of Japanese quail dosed orally with aroclor 1254 at a rate of 7 mg/kg per day.

Duration of dosing and treatment	Quail	liver weight / body weight x 100	thymus weight (grams)
4 weeks			
Control	1	3.1	0.0400
	2	2.1	0.0519
	3	3.2	0.0799
	4	5.4	0.0532
	5	3.2	0.0817
	Mean ± S.D.	3.4 ± 1.2	0.0614 ± 0.0185
Aroclor 1254	1	4.3	0.1099
	2	3.8	0.0719
	3	3.6	0.0930
	4	3.3	0.1290
	5	2.5	0.0468
	Mean ± S.D.	3.5 ± 0.7	0.0901 ± 0.0321
8 weeks			
Control	1	2.4	0.0151
	2	1.8	0.0921
	3	2.6	0.0685
	4	2.7	0.0630
	5	1.2	0.0207
	Mean ± S.D.	2.2 ± 0.5	0.0519 ± 0.0330
Aroclor 1254	1	2.0	0.0315
	2	1.7	0.0268
	3	2.2	0.1419
	4	2.0	0.0155
	5	2.2	0.0078
	Mean ± S.D.	2.0 ± 0.2	0.0447 ± 0.0551
12 weeks			
Control	1	1.9	0.0183
	2	2.5	0.0213
	3	2.2	0.0108
	Mean ± S.D.	2.2 ± 0.3	0.0168 ± 0.0054
Aroclor 1254	1	2.5	0.0475
	2	2.4	0.0945
	3	2.5	0.0752
	4	1.7	0.0124
	5	2.5	0.04496
	Mean ± S.D.	2.3 ± 0.4	0.0556 ± 0.0311

TABLE 3.10. Liver and thymus weights of quail dosed orally with PCB congeners. Means which are significantly different from controls are followed by the significance level.

Duration of dosing treatment and dose	Quail	liver weight / body weight X 100	thymus weight (grams)
2 weeks			
Corn oil	1	3.1	0.0400
	2	2.1	0.0519
	3	3.2	0.0799
	4	5.4	0.0532
	5	3.2	0.0817
	Mean ± S.D.	3.4 ± 1.2	0.0614 ± 0.0185
153, 4 mg/kg/day	1	3.5	0.0744
	2	3.4	0.0931
	3	3.0	0.0757
	4	3.2	0.1038
	5	3.7	0.0834
	6	2.2	0.0795
	Mean ± S.D.	3.2 ± 0.5	0.0850 ± 0.1141
105, 3 mg/kg/day	1	2.9	0.2903
	2	3.6	0.0478
	3	2.2	0.1987
	4	3.6	0.0363
	5	2.9	0.0441
	6	3.1	0.0800
	Mean ± S.D.	3.0 ± 0.5	0.1162 ± 0.1048
# 126	1	2.9	0.0236
0.05 mg/kg/day	2	3.6	0.0639
	3	4.3	0.0251
	4	4.4	0.0565
	5	2.6	0.0463
	6	3.3	0.0313
	Mean ± S.D.	2.9 ± 0.5	0.0410[a] ± 0.0170
8 weeks			
Corn oil	1	2.4	0.0151
	2	1.8	0.0921
	3	2.6	0.0685
	4	2.7	0.0630
	5	1.7	0.0207
	Mean ± S.D.	2.2 ± 0.5	0.0519 ± 0.0330
153, 4 mg/kg/day	1	2.3	0.1148
	2	2.2	0.0108
	3	3.0	0.0060
	4	2.1	0.0473
	5	3.4	0.0570
	Mean ± S.D	2.6 ± 0.6	0.0472 ± 0.0439

Table 3.10, contin.

105, 3 mg/kg/day	1	1.8	0.0275
	2	2.7	0.0486
	3	2.8	0.0503
	4	2.4	0.0488
	Mean ± S.D.	2.4 ± 0.5	0.0438 ± 0.0109
126	1	3.0	0.0
0.05 mg/kg/day	2	1.6	0.0994
	3	3.0	0.1249
	4	2.4	0.0442
	5	2.2	0.0375
	Mean ± S.D.	2.3 ± 0.6	0.0620 ± 0.0450

a - $p < 0.05$

4. ACUTE DOSING STUDY

4.1 Objectives

The objectives of this experiment were:

1) to examine the dose response relationship between single doses of Aroclor 1254 and porphyria and PSMO induction in avian liver and kidney;

2) to compare the effects (porphyria, PSMO induction) among two non-ortho PCB congeners (#s 126 and 77) and a di-ortho PCB congener, # 153.

Aroclor 1254 was given in three doses, 100, 250 and 500 mg/kg. Two PCB congeners, # 153 and # 77, were given to quail at a dose of 250 mg/kg (the medium dose used in the Aroclor study). A third congener, # 126, was given at a lower dose, 25 mg/kg, due to concerns over possible acute toxicity at 250 mg/kg.

4.2 Results

4.2.1 Liver and kidney porphyrin levels

4.2.1.1 Aroclor 1254 dosed birds

Porphyrin levels expressed as a percent of control values in tissues of birds dose with Aroclor 1254 are shown in Fig. 4.1. Porphyrin levels in each bird are in Table 4.1. Aroclor 1254 increased liver HCP levels significantly at all doses. HCP accumulation among individual birds was very variable, especially in the group dosed with 250 mg/kg of Aroclor 1254, where HCP levels ranged from 77 pmols/g to 3616 pmols/g. Kidney HCP levels in birds dosed with Aroclor 1254 had a similar pattern to liver, but with greater variability among the birds dosed with 250 mg/kg/day, 66 - 30,117 pmols/g.

Aroclor 1254 caused a highly significant and dose-related increase in COPRO levels in liver but not in kidney. Aroclor 1254 caused a highly significant and dose-related increase in PROTO levels in liver

but not in kidney.

4.2.1.2 Congener dosed birds

Porphyrin levels expressed as a percent of the control values are presented in Fig. 4.2. Chromatograms which illustrate the liver porphyrin pattern in a quail dosed with PCB 126 versus one dosed with cornoil are shown in Fig. 4.3. Porphyrin levels in individual birds are in Table 4.1. PCB 126 caused a highly significant increase in liver and kidney HCPs, averaging 103-fold in liver and 123-fold in kidney. PCB 77 did not change HCP levels in liver or kidney. Liver porphyrin levels in birds dosed with PCB 153 were elevated 4.4-fold, although the difference was not significant. Three of four birds in this group had HCP levels which overlapped with controls, while one bird had liver HCP levels of 359 pmols/g. Kidney HCP levels in birds dosed with PCB 153 were highly correlated with those in livers, although the mean level in kidney was significantly higher than controls.

Both # 126 and # 153, but not # 77, caused significant elevation of hepatic and renal COPRO levels. Liver levels of PROTO were significantly elevated in birds dosed with PCB 153 but not in those dosed with PCBs 126 and 77. Renal PROTO levels were not altered significantly by any of the PCB congeners.

4.2.2 PCB Residue Levels

Birds given 500 mg/kg of Aroclor 1254 had significantly higher residue levels in liver but not in kidney than birds given lesser doses or corn oil (Table 4.2). Levels of PCB 153 were much higher but very variable in livers than in kidney pools. Trace amounts of PCB 77 were found in liver with slightly higher levels in kidney. Levels of PCB 126 were higher in liver than in kidney.

Fig. 4.1 Comparison of the effects of Aroclor 1254 on hepatic and renal porphyrin levels in groups of Japanese quail given single oral doses of PCBs or corn oil and killed after 5 days (See Methods for details of dosing of birds). The mean values for each group (N = 4) are plotted as percentages of the mean control values (N = 4).

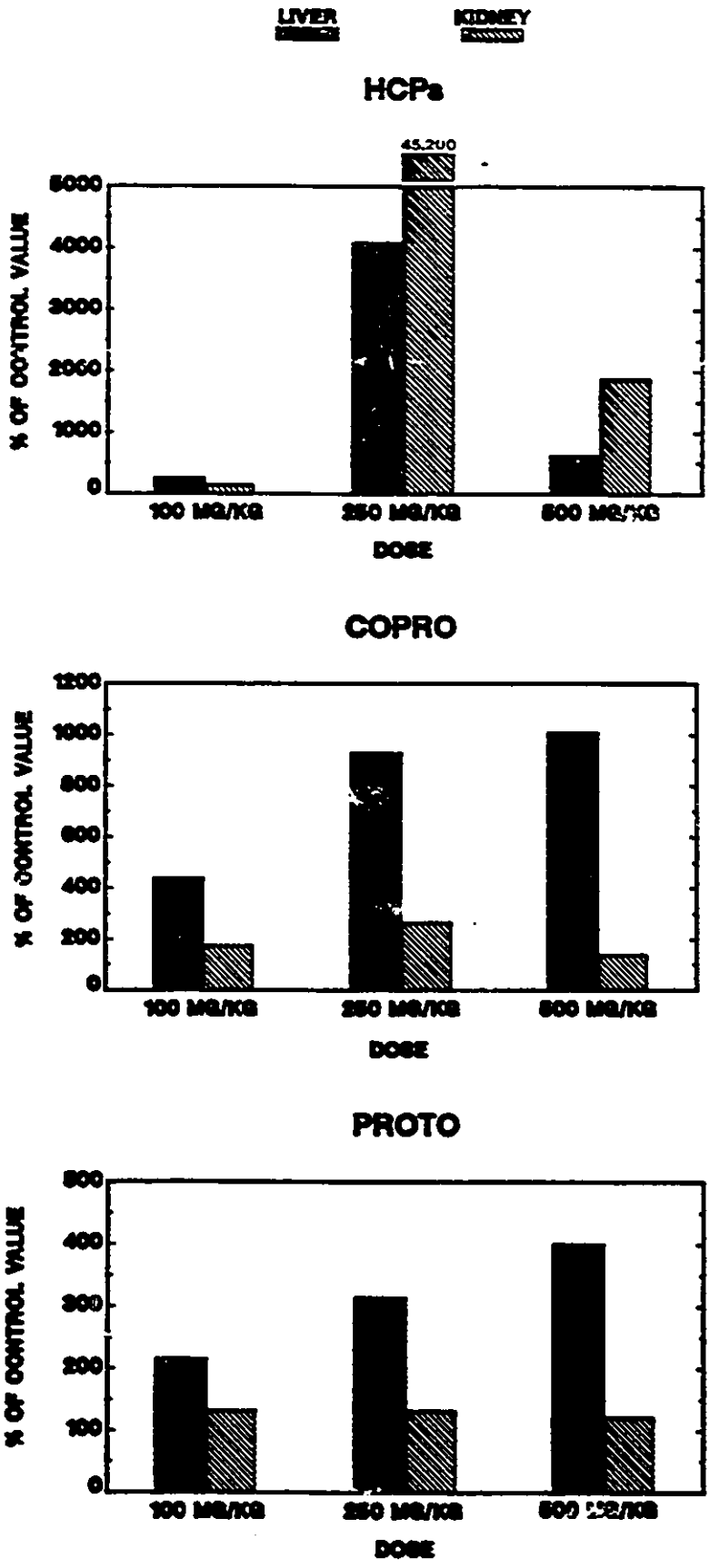

LIVER
KIDNEY
HCPs
45,200
5000
4000
3000
2000
1000
0
% OF CONTROL VALUE
100 MG/KG
250 MG/KG
500 MG/KG
DOSE
COPRO
1200
1000
800
600
400
200
0
% OF CONTROL VALUE
100 MG/KG
250 MG/KG
500 MG/KG
DOSE
PROTO
500
400
300
200
100
0
% OF CONTROL VALUE
100 MG/KG
250 MG/KG
DOSE

Fig. 4.2 Comparison of the effects of three PCB congeners on hepatic and renal porphyrin levels in groups of Japanese quail given single oral doses and killed after 5 days (See Methods for details of dosing). The mean values for each group (N = 4) are plotted as percentages of the mean control values (N = 4).

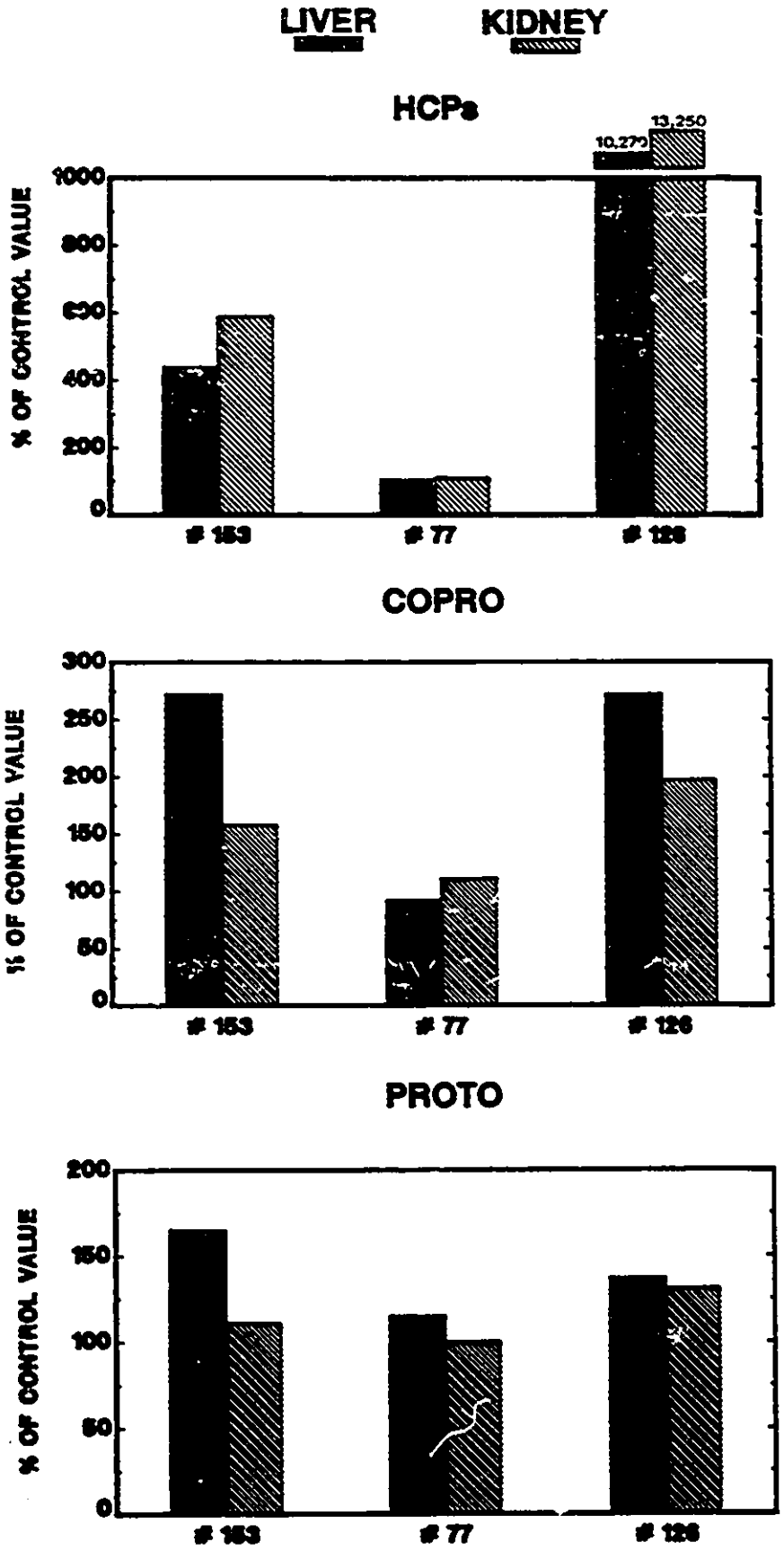
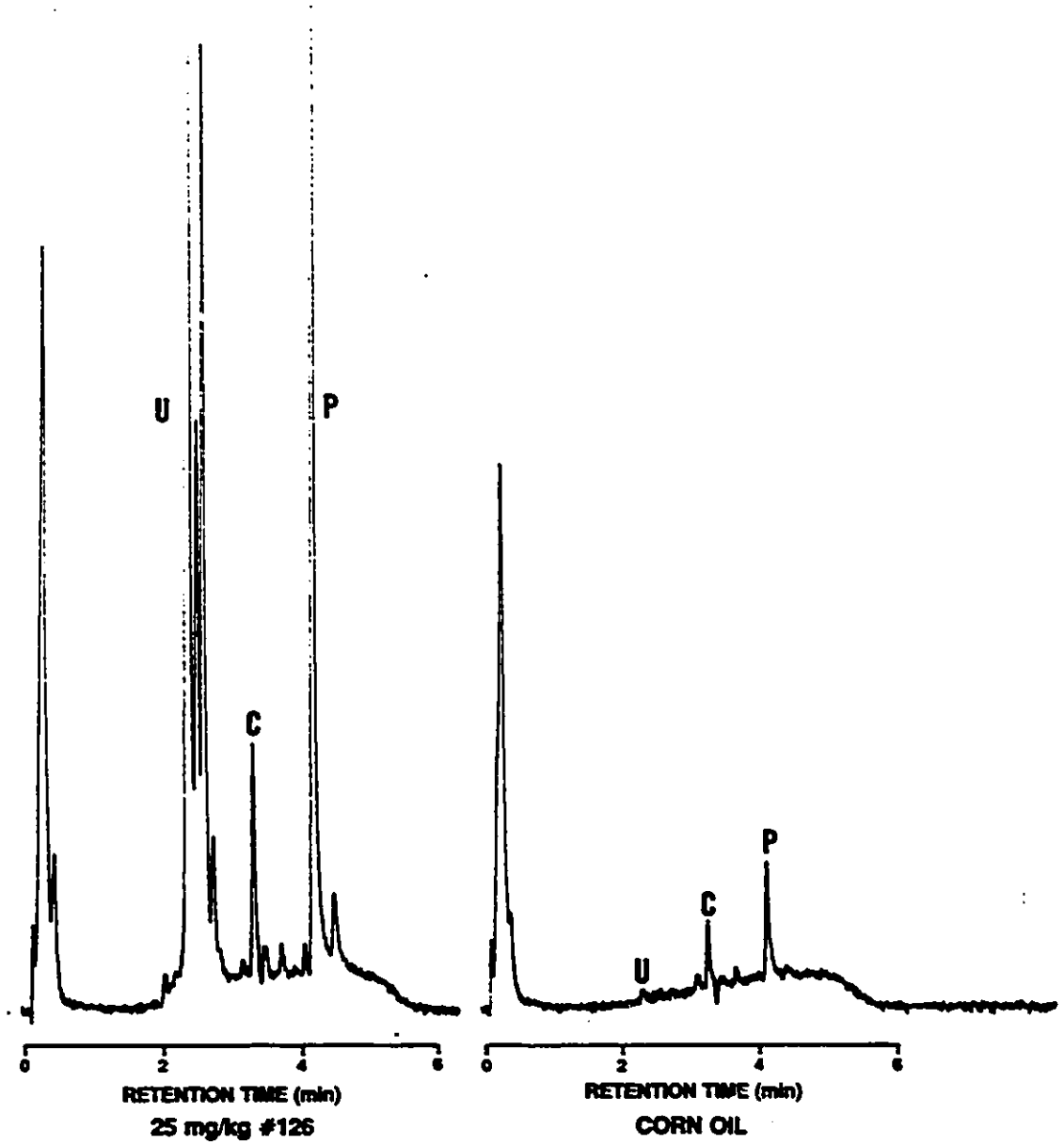

LIVER
KIDNEY
HCPs
10,270
13,250
% OF CONTROL VALUE
1000
800
600
400
200
0
153
77
126
COPRO
% OF CONTROL VALUE
300
250
200
150
100
50
0
153
77
126
PROTO
% OF CONTROL VALUE
200
150
100
50
0
153
77
126

Fig. 4.3 HPLC Chromatograms which compare the increase in hepatic porphyrin levels in a Japanese quail given a single 25 mg/kg oral dose of PCB 126 compared to a bird given corn oil. Both birds were killed after 5 days (See Methods for details of dosing of birds). (U = uroporphyrin, C = coproporphyrin, P = protoporphyrin).


U
P
C
0
2
4
6
RETENTION TIME (min)
25 mg/kg #126
U
C
P
0
2
4
6
RETENTION TIME (min)
CORN OIL

4.2.3 Liver, kidney polysubstrate monooxygenase activity

PSMO activity levels expressed as a percent of the control values are in Figs. 4.4 and 4.5. PSMO activity levels in each bird are in Table 4.3.

4.2.3.1 Aroclor 1254 dosed birds

Aroclor 1254 increased hepatic EROD activity in a highly significant manner, which was not dose-related, 25-fold at 100 mg/kg, 23-fold at 250 mg/kg and 18-fold at 500 mg/kg (Table 4.5). In contrast kidney EROD activity was increased by Aroclor 1254 in a dose-related fashion.

Aroclor 1254 increased hepatic APND activity, which was highest (2.8-fold) in birds dosed with 250 mg/kg (Table 4.4). APND activity in kidney was increased by Aroclor 1254, with the greatest increase occurring at 500 mg/kg (4.8-fold).

4.2.3.2 Congener dosed birds

PCB 126 increased EROD activity 17-fold in liver and 3.3-fold in kidney. PCB 153 increased EROD activity 16-fold in liver and 1.8-fold in kidney. PCB 77 caused a slight (1.8-fold) but significant increase in hepatic EROD activity and a decrease in renal EROD activity.

PCB 126 increased APND activity 2.8-fold in liver and 2.5-fold in kidney. PCB 153 increased APND activity 2.6-fold in liver and 3.1-fold in kidney. PCB 77 did not affect APND activity in either liver or kidney.

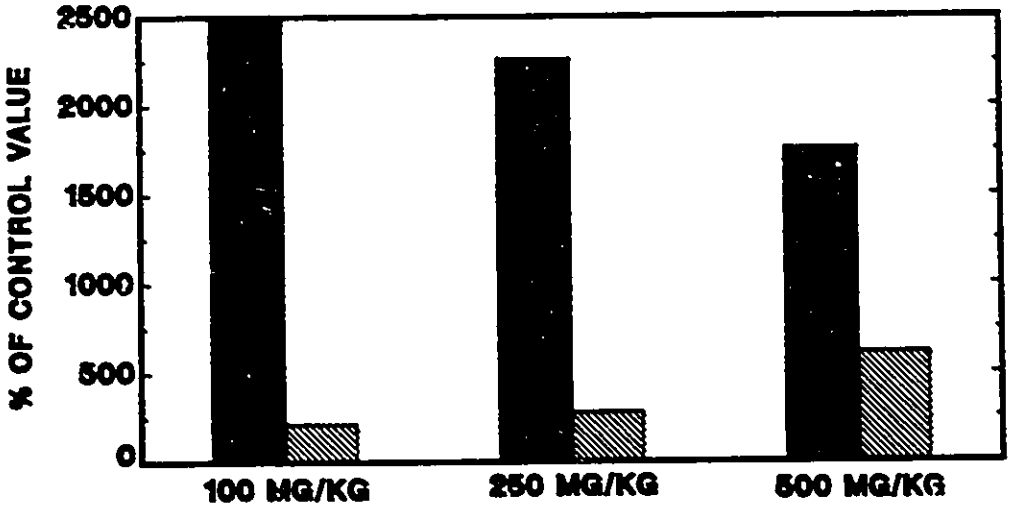
4.2.4 Liver and bursa weights

Aroclor 1254 increased in liver weight significantly at all doses (Table 4.4). PCB 153 also increased liver weight significantly. There were no differences in bursa weights between dosed or control birds.

Fig. 4.4 Effects of Aroclor 1254 EROD and APND activity levels in liver and kidney of groups of Japanese quail given single oral doses of PCB or corn oil and killed after 5 days (See Methods for details of dosing). The mean values for each group (N = 4) are plotted as percentages of the mean control values (N = 4).

LIVER KIDNEY

EROD



APND

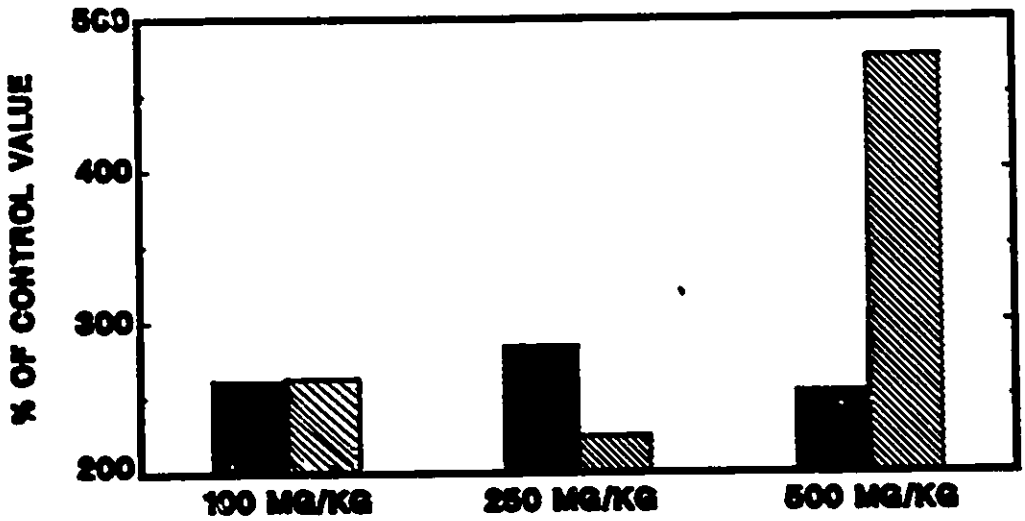
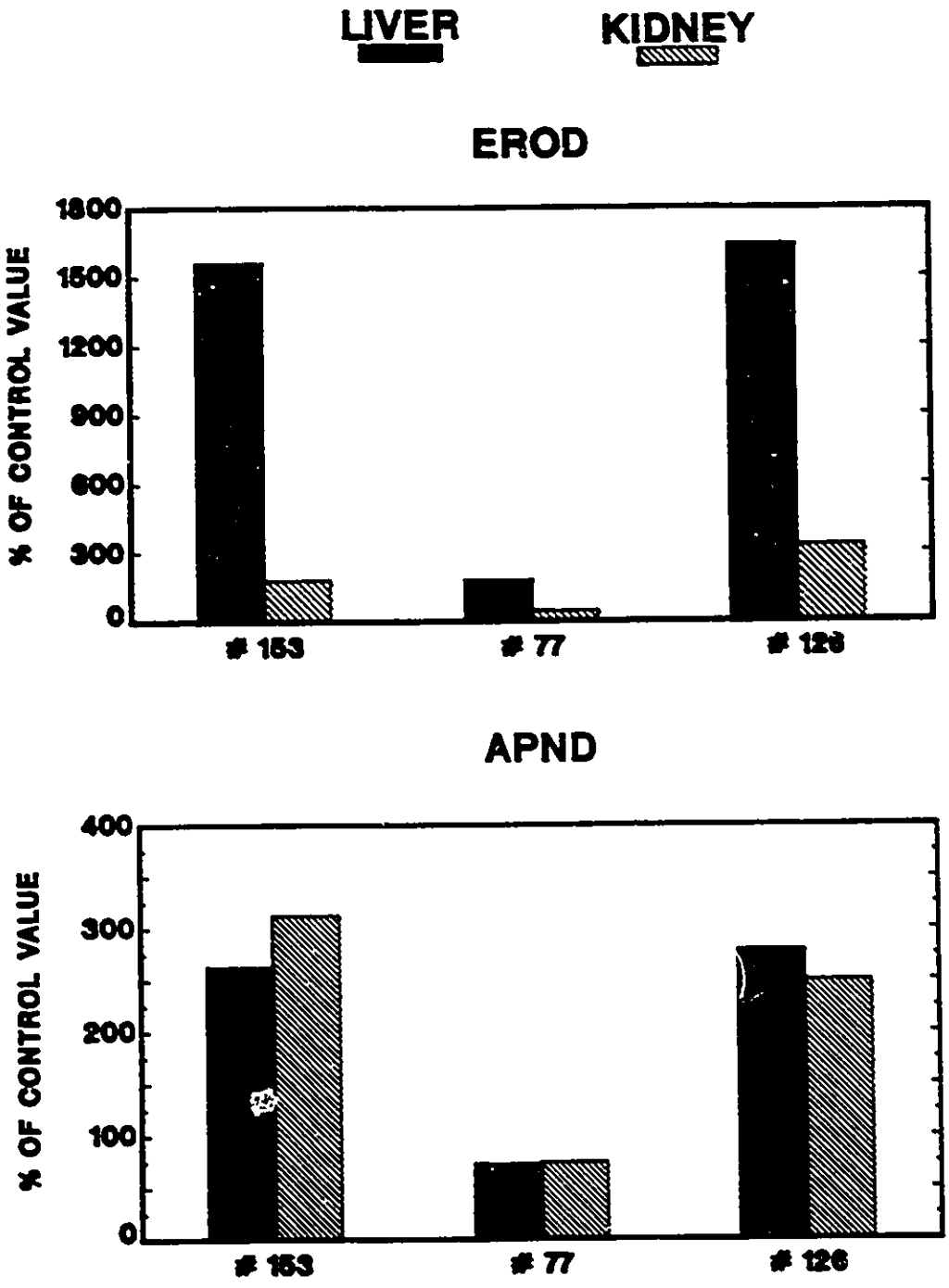


Fig. 4.5 Effects of three PCB congeners on EROD and APND activity levels in liver and kidney of groups of Japanese quail given single oral doses of PCBs or corn oil and killed after 5 days (See Methods for details). The mean values for each group (N = 4) are plotted as percentages of the mean control values (N = 4).


LIVER
KIDNEY
EROD
% OF CONTROL VALUE
1800
1500
1200
900
600
300
0
153
77
128
APND
% OF CONTROL VALUE
400
300
200
100
0
153
77
126

4.3 Discussion

4.3.1 Effects of PCBs on porphyrin levels

Aroclor 1254 caused significant accumulation of hepatic and renal porphyrins (HCPs, COPRO and PROTO) within five days of a single dose. Over a range of 100 to 500 mg/kg the response was dose-related for COPRO and PROTO but not for HCPs in liver and for none of the porphyrin compounds in kidney. Miranda *et al.* (1987) reported that over the same dosage range that Aroclor 1242 caused a dose-dependent increase in total porphyrins in liver and small intestine of male Japanese quail, within 48 hours after dosing. In another study, Miranda *et al.* (1986) reported that 500 mg/kg Aroclor 1254 caused total porphyrin accumulation of 1700-fold in kidney and 76-fold in liver of Japanese quail and that porphyrin accumulation peaked 48 hours after an acute dose of Aroclor. Birds in Table 4.1 were killed 5 days after dosing, so HCP levels may have been even higher a few days earlier.

The accumulation of HCPs in liver and kidney is characterized by extreme variability among individual birds in responsive groups (Table 4.1). There was a 47-fold difference in liver and 456-fold difference in kidney between the highest and lowest individuals among birds dosed with 250 mg/kg Aroclor 1254. Liver PCB residue levels do not indicate a lack of chemical being absorbed and reaching the target organ, as the quail with the highest porphyrin levels had a liver concentration of 5.2 mg/kg and the bird with the lowest liver HCP levels had 5.3 mg/kg of Aroclor 1254 present in its liver. EROD activity levels were nearly identical in the two birds, while APND activity was about 2-fold higher in the bird with the minimal accumulation of porphyrins. Among the quail dosed with PCB 126, three of four birds showed extreme

accumulation of liver and kidney porphyrins, while a fourth bird was only marginally different from controls. This fourth bird had the highest liver residues of PCB 126, while it had slightly lower EROD, but higher APND activity than other birds in its group.

HCP accumulation in sensitive species such as Japanese quail and rats appears to be exponential in response to acute doses of PHAHs (Kennedy, 1988). This exponential increase in porphyrins could be explained by the model proposed by Bonkovsky et al. (1987). They suggest that the main mechanism of HCP accumulation involves oxidation of porphyrinogens to porphyrins which then further inhibit the activity of uroporphyrinogen decarboxylase, which in turn leads to more accumulation of uroporphyrinogen and therefore of uroporphyrin. Kinetic aspects of this process may also underlie the wide difference in individual response observed in my experiments. Such a rapid exponential increase in porphyrin accumulation would tend to exaggerate the difference between responsive and non-responsive individuals especially when small sample sizes are used. Methods of measuring porphyrin levels at short intervals without killing animals, possibly by measuring porphyrins in blood, would permit closer study of the kinetics of the response. Larger sample sizes should also be used.

4.3.1.1 Comparison among congeners

PCB 126 caused considerable accumulation of HCPs in liver and kidney after a single 25 mg/kg dose. In contrast, 250 mg/kg PCB 77, another of the non-ortho substituted PCBs, caused no effect on liver or kidney porphyrins. PCB # 153 at 250 mg/kg caused clear HCP accumulation in only one of four birds. Only trace amounts of # 126 and even less of # 77 were detected in both tissues. In contrast, # 153 produced mean

liver residue levels three to four orders of magnitude higher than the other two PCBs.

PCB 77 did not accumulate in mouse liver (Sano et al., 1985), while, Leece et al (1985) attributed the low toxicity of # 77 in rats to rapid metabolism. However, if # 77 was rapidly metabolized in quail liver, it was via a pathway not characterized by EROD or APND.

PCB 77 has been shown to cause porphyrin accumulation in chick embryo liver cell culture in a number of studies (Sano et al., 1985; Sassa et al., 1986; Marks et al., 1982). However, when injected into chicken eggs # 77 caused only minor porphyrin accumulation, considered to be mainly COPRO, at higher doses (Rifkind et al., 1985), although in that same study, neither TCDD nor PCB 169 (3,3'4,4'5,5'-HCB) caused significant porphyrin accumulation in chick embryos even at a dose of 1000 nmol/egg. Miranda et al. (1987) administered PCB 77 orally in corn oil at a dose of 87.6 mg/kg and found that it caused a significant increase in liver total porphyrins within 48 hours, although the increase was less than that caused by PCB 47 (2,2',4,4'-TCB) given at the same dosage.

The wide difference in effects of PCB 77 between Miranda et al. (1987) and the those results in Tables 4.1 and 4.3 is puzzling. The chemical used in their experiments was purchased from the same supplier (Foxboro/Analabs/Ultra Scientific). They analyzed their compound by GC/MS and found no evidence of trace impurities such as PCDDs or PCDFs. The difference may be sex related. Cecil et al. (1975) reported differences in phenobarbitol sleeping times between male and female quail dosed with Aroclor 1254. However, the difference in results between the two studies may rather be a function of intestinal

absorbance related to the solubility of the PCB congener. Because of solubility problems (see Methods for details), PCB 77 was administered to quail as a suspension in corn oil instead of in solution, as were the other compounds. Miranda et al. (1987) used a lower dose (80 mg/kg) "in corn oil" and therefore presumably dissolved. Kennedy (1988) reported that HCB dissolved in corn oil caused a much more rapid uptake and higher total HCB residues in liver, kidney and spleen than if the chemical was administered as a powder.

4.3.1.2 Liver and kidney porphyrins

Comparison of the dose-response in liver versus kidney shows that at 100 mg/kg HCPs are higher in liver, but that at 250 mg/kg they are much higher in kidney which continues at 500 mg/kg. High levels of porphyrins in kidney may originate in situ or they may be transferred from liver to kidney by the circulatory system. Miranda et al. (1986) reported that Japanese quail dosed with 500 mg/kg Aroclor 1254 had greater inhibition of renal than hepatic uroporphyrinogen decarboxylase, thus indicating that some of the porphyrins in kidney originate there. They also found minimal amounts of HCPs in quail feces, suggesting an inability to eliminate porphyrins, which may explain their extreme accumulation. In contrast, rats and mice can eliminate large amounts of HCPs in urine (Goldstein et al., 1974; Cantoni et al., 1981).

Miranda et al. (1987) reported that Aroclor 1254 increased the activity of ALA-S to a greater degree in quail kidney than in liver. An increase in ALA-S activity may cause accumulation of uroporphyrinogen and of its oxidation product, uroporphyrin (Cantoni et al, 1987). Increased ALA-S activity can result from an increased cellular demand for heme caused by induction of the cytochrome P-450 MFOs. The activity

level of both EROD and APND in Table 4.2 was lower in kidney than in liver. EROD was induced to a much greater extent, 25-fold maximum in liver and 6.2-fold maximum in kidney, while APND was induced 2.8-fold maximum in liver versus 4.8-fold in kidney. However, both enzymes were maximally induced in liver at 100 mg/kg of Aroclor 1254, while there was a dose related increase in activity especially for EROD in kidney. The increased demand for more heme for P-450 synthesis may therefore contribute to greater HCP accumulation in kidney than liver at higher doses.

4.3.2 Induction of polysubstrate monooxygenases

Maximal induction of hepatic EROD occurred at 100 mg/kg of Aroclor 1254. Both # 126 and # 153 increased EROD to a degree comparable to 500 mg/kg Aroclor 1254. For PCB 126, this was expected as this compound is known to be a potent P-450-MC type inducer (Leece et al, 1985). Potent EROD induction by PCB 153 was not however expected. A sample of # 153 was analyzed (by Dr. R.J. Norstrom). It contained a variety of TCDD and TCDF contaminants at trace levels, in particular 1.25 ug/g of 2378-TCDD

Levels of TCDDs and TCDFs in commercially acquired "pure" PCB 153 (2,2'4,4,5,5'-HCB) as analyzed by GC-MS.

Compound	Concentration (ng/g)
1368-TCDD	915
1378-TCDD	127
2378-TCDD	1254
12378-PCDD	121
123478-HxCDD	566
1234678-HpCDD	270
2378-TCDF	775

Quail dosed with PCB 153 would have received approximately 313 ng/kg dose of TCDD. As little as 6 nmols/egg of TCDD causes maximal EROD induction when injected into chick embryos (Rifkind et al., 1985). However, in that same study , TCDD did not cause any porphyrin accumulation in chick embryo livers when injected into eggs at a range from 0.1 to 12 nmols/egg. TCDD causes uroporphyrin accumulation in chick embryo cell cultures (Lambrecht et al., 1988). However, other than in chick embryo cell culture, PCDDs and PCDFs have not been shown to cause porphyria in birds. Rifkind et al. (1984) attributed hepatic P-448 type induction in chick embryos dosed with # 153 to contamination by trace amounts of 2378-TCDF. However, Goldstein et al. (1976) showed that 2378-TCDF, at 5 ug/kg did not cause porphyria in chicks and therefore is unlikely to have been the cause of porphyria as a result of dosing with # 153 containing trace amounts of TCDF.

Although it is possible that the EROD induction and porphyria observed in birds dosed with PCB 153 were in fact caused by the trace amounts of TCDD and other contaminants including the TCDF, there is no evidence to indicate that the trace contaminants would account for the porphyrin accumulation. It is also interesting that # 153 caused hepatic and renal EROD and APND induction on par with Aroclor 1254 and PCB 126, but that HCPs were elevated in only one bird thus indicating a lack of association between PSMO induction and HCP accumulation.

Significant induction of P-450-PB type enzymes by # 153 is consistent with it having been characterized as a P-450-PB inducer in rats (Stonard & Grieg, 1976), although in chick embryo liver, # 153 caused only slight APND induction even at 5000 nmols/egg (Rifkind et al., 1984).

With the exception of PCB 77, all the compounds and doses tested caused similar degree of hepatic APND induction. The strong induction of APND by PCB 126 was surprising, as this compound did not induce a P-450-PB type response in rats (Leece et al., 1985). There are other reports of coplanar PCB compounds inducing strong P-450-PB type responses in birds. Rifkind et al. (1985) reported that # 77 (500 nmols/egg) and # 169 (50 nmols/egg) caused significant APND induction in chick embryo livers. Jones et al. (1985) also reported significant APND induction in chickens by PCB 77.

A brief review of the literature on enzyme induction in birds indicates that they do not necessarily respond in the same manner as laboratory rats. There are also many differences among species. Phenobarbitol induced a strong P-450-PB type response in chick embryo liver (Rifkind et al., 1982). However, PB did not induce APND activity in Japanese quail (Sifri et al., 1975; Buckpitt & Boyd, 1982; Carpenter et al., 1985) or in ducks (Sifri et al., 1975). Also 3-methylcholanthrene did not induce P-450-MC type activity as characterized by EROD in Japanese quail (Neal et al., 1986); they also reported wide difference in metabolism of Aflatoxin B_1 between rats and quail.

Studies with other birds, especially non-domestic species show a variety of PSMO responses to PCB dosing. European buzzards (Buteo buteo) dosed with DP_5 (a PCB mixture similar to Aroclor 1254) showed no increase in hepatic cytochrome P-450 or in activity of a number of PSMO enzymes, including EROD; whereas, Japanese quail responded with significant increases in cytochrome P-450 and EROD and ECOD activity

(Riviere et al, 1985). In contrast, Rinzky & Perry (1983) reported that Aroclor 1254 given at 10 mg/kg for 7 days caused significant AE and APND induction in Barn Owls (Tyto alba) but not in chickens. However, it should be noted that the extreme variability in the activity of AE in dosed chickens may have masked a significant increase in activity. American kestrels (Falco sparvarius) dosed with 7 mg/kg/day of Aroclor 1254 for 4, 8 and 12 weeks and with up to 500 mg/kg of Aroclor 1254 and sacrificed after 5 days showed no significant increase in hepatic EROD activity in contrast to that reported for quail in this thesis. Also PCB # 126 at a dose of 0.05 mg/kg/day for 4 weeks increased AE activity 5.3-fold, whereas # 153, at a dose of 4 mg/kg/day increased AE activity only 2.2-fold (Elliott & Kennedy, unpub. data).

4.3.3 Liver and bursa weights

Aroclor 1254 at all doses and PCB 153 caused significant increases in liver weight, while PCB #s 77 and 126 did not. Therefore, there was no indication of an association between increase in liver weight and porphyrin or PSMO induction. Nikolaides et al. (1988) reported that PCB 77 caused a reduction in dry bursal weight in chick embryos. No significant changes in fresh bursal weight were observed in quail given various PCBs shown in Table 4.4.

4.4 Conclusions

PCB 126 caused extreme accumulation of highly carboxylated porphyrins and was a potent inducer of both P-450-MC and -PB type polysubstrate monooxygenases in female Japanese quail. In contrast PCB 77 caused only slight EROD induction.

Effects observed for PCB 153 must be considered in light of the contamination of the commercially acquired chemical with significant

levels of dioxins and furans. Commercially acquired PCB congeners should be tested independently prior to use in toxicology studies. Lack of any marked effects by PCB 77 may relate to its lower solubility in organic solvents and therefore lower absorption by the intestine and other cellular membranes.

The polysubstrate monooxygenase system of the Japanese quail is different from that of the rat. Further studies with the classic inducing compounds should be conducted in a variety of bird species in order to further characterize their drug metabolizing enzyme systems.

Renal accumulation of highly carboxylated porphyrins was more marked than hepatic accumulation in quail given single high doses of PCBs. Therefore, the kidney may be a useful tissue for biomonitoring of porphyrin levels, especially in acute exposure situations.

TABLE 4.1 Porphyrin levels in liver and kidney of Japanese quail dosed orally with Aroclor 1254 or PCB congeners. Experimental groups for which the means are significantly different (t-test) from controls are followed by the significance level.

Treatment and dose	Quail	HCPs (pmols/g)		Copro-porphyrin (pmols/g)		Proto-porphyrin (pmols/g)	
		Liver	Kidney	Liver	Kidney	Liver	kidney
Aroclor 1254							
100 mg/kg	1	31	21	294	51	232	81
	2	48	10	287	76	127	69
	3	52	15	344	45	299	95
	4	113	63	248	94	134	99
Mean ± S.D.		61[a] ± 36	27 ± 24	293[c] ± 39	67[b] ± 23	198[b] ± 83	86 ± 14
250 mg/kg	1	119	2356	996	87	284	71
	2	3616	30117	792	139	328	73
	3	77	438	279	88	328	83
	4	105	66	420	90	217	116
Mean ± S.D.		980[b] ± 1758	8244[b] ± 14616	622[c] ± 330	101[c] ± 25	289[c] ± 52	86 ± 21
500 mg/kg	1	262	575	1010	76	612	53
	2	192	53	543	37	326	75
	3	29	209	433	53	212	82
	4	111	513	717	46	331	107
Mean ± S.D.		149[b] ± 101	337[c] ± 248	675[c] ± 251	53 ± 17	370[c] ± 170	79 ± 22

Table 4.1, contin.

153, 250 mg/kg	1	38	47	290	45	161	58
	2	350	329	191	93	168	93
	3	9	20	107	49	181	59
	4	21	27	138	53	98	79
Mean ± S.D.		105 ± 164	106[a] ± 149	182[b] ± 80	60[a] ± 22	152[b] ± 37	72 ± 17
77, 250 mg/kg	1	11	20	71	32	83	51
	2	25	27	68	30	137	63
	3	22	14	71	45	111	63
	4	42	14	48	63	93	81
Mean ± S.D.		25 ± 13	20 ± 7	65 ± 11	42 ± 19	106 ± 24	65 ± 15
126, 25 mg/kg	1	4000	4070	156	52	127	141
	2	38	225	104	104	126	105
	3	3901	3667	179	92	159	550
	4	1920	1771	168	50	92	38
Mean ± S.D.		2465[c] ± 1880	2385[c] ± 1861	182[c] ± 30	75[b] ± 28	126 ± 27	85 ± 47
Corn oil	1	40	29	81	39	63	63
	2	43	17	73	35	125	86
	3	21	22	77	46	87	52
	4	19	12	68	30	92	43
	5	20	16	77	36	81	78
	6	18	19	30	48	114	57
	7	7	10	63	34	80	79
Mean ± S.D.		24 ± 13	18 ± 6	67 ± 17	38 ± 7	92 ± 21	65 ± 16

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

TABLE 4.2 PCB residue levels in liver (mean, S.D.) and kidney (pooled samples) of Japanese quail given a single oral dose of Aroclor 1254 or PCB congeners and sacraficed after 5 days. N = 4, except for controls, where N = 7.

Treatment and dose	Percent water		Residue level (mg/kg, wet weight)	
	liver	kidney	liver	kidney
Control	71	74	nd	nd
Aroclor 1254				
100 mg/kg	70 ± 1.0	69	6.5 ± 2.5	5.1
250 mg/kg	71 ± 0.8	73	6.0 ± 2.0	6.5
500 mg/kg	71 ± 0.6	73	21 ± 9.0	6.8
153, 250 mg/kg	64 ± 4.9	80	141 ± 182	17.4
77, 250 mg/kg	71 ± 1.6	71	0.01 ± 0.01	0.04
126, 25 mg/kg	67 ± 0.8	75	0.09 ± 0.07	0.05

nd - none detected

TABLE 4.3 Microsomal total protein and monooxygenase activities in liver (mean, S.D.) and kidney (pooled samples) of Japanese quail dosed orally with Aroclor 1254 or PCB congeners. Experimental groups which are significantly different (t-test) from controls are followed by the significance level.

Treatment and dose	Quail	Total protein (ug/ml)		7-ethoxyresorufin O-deethylase (nmols/mg/min)		Aminopyrine N-demethylase (nmols/mg/min)	
		Liver	kidney	liver	kidney	liver	kidney
Aroclor 1254							
100 mg/kg	1	3.7		1.66		7.6	
	2	3.1		1.26		10.0	
	3	3.7		0.63		8.3	
	4	3.3		1.44		13.1	
	Mean ± S.D.	3.5 ± 0.3	1.7	1.25[c] ± 0.44	0.13	9.8[b] ± 2.5	2.1
250 mg/kg	1	2.9		1.19		10.0	
	2	3.6		0.76		11.2	
	3	2.8		1.57		11.4	
	4	4.8		1.01		9.7	
	Mean ± S.D.	3.5 ± 0.9	1.2	1.13[c] ± 0.34	0.17	10.6[c] ± 0.9	1.8
500 mg/kg	1	5.0		0.67		8.5	
	2	4.8		0.68		9.6	
	3	4.2		1.02		7.4	
	4	4.2		1.15		12.3	
	Mean ± S.D.	4.6 ± 0.4	1.5	0.88[c] ± 0.24	0.37	9.5[2] ± 2.1	3.8

Table 4.3, contin.

153, 250 mg/kg	1	2.7		1.07		14.5		
	2	3.4		0.81		14.9		
	3	3.1		0.90		3.9		
	4	2.9		0.35		5.9		
	Mean ± S.D.	3.0 ± 0.3	1.7	0.78[c] ± 0.31	0.11	9.8[1] ± 5.7	2.5	
77, 250 mg/kg	1	1.8		0.09		4.4		
	2	2.7		0.10		3.7		
	3	3.4		0.10		0.7		
	4	3.0		0.05		2.1		
	Mean ± S.D.	2.7 ± 1.7	1.1	0.09[a] ± 0.02	0.03	2.7 ± 1.7	0.6	
126, 25 mg/kg	1	2.7		0.74		11.1		
	2	3.7		1.23		8.4		
	3	2.5		0.43		10.5		
	4	2.4		0.88		11.6		
	Mean ± S.D.	2.8 ± 0.6	1.3	0.82[c] ± 0.33	0.20	10.4[c] ± 1.4	2.0	
Corn oil	1	4.3		0.03		2.0		
	2	4.4		0.04		4.3		
	3	3.6		0.03		2.5		
	4	1.9		0.05		3.2		
	5	3.5		0.06		2.8		
	6	2.2		0.06		7.4		
	7	2.9		0.05		3.9		
	Mean ± S.D.	3.3 ± 1.0	1.5	0.05 ± 0.01	0.06	3.7 ± 1.8	0.8	

[1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.001$

TABLE 4.4 Liver and bursa weights of Japanese quail dosed orally with aroclor 1254 or PCB congeners. Experimental groups which are significantly different (t-test) from controls are followed by the significance level.

Treatment and dose	Quail	liver weight / body weight X 100	bursa weight (grams)
Aroclor 1254			
100 mg/kg	1	2.1	0.06
	2	2.4	0.08
	3	2.3	0.05
	4	3.3	0.09
Mean ± S.D.		2.5[a] ± 0.53	0.07 ± 0.02
250 mg/kg	1	2.9	0.18
	2	3.1	0.07
	3	2.7	0.06
	4	2.4	0.08
Mean ± S.D.		2.8[c] ± 0.30	0.10 ± 0.06
500 mg/kg	1	2.5	0.06
	2	2.8	0.02
	3	2.5	0.09
	4	2.2	0.10
Mean ± S.D.		2.5[b] ± 0.24	0.07 ± 0.04

Table 4.4, contin.

153, 250 mg/kg	1	2.9	0.16
	2	2.7	0.21
	3	2.6	0.06
	4	2.5	0.02
Mean ± S.D.		2.7[c] ± 0.17	0.11 ± 0.09
77, 250 mg/kg	1	2.4	0.08
	2	2.0	0.09
	3	2.0	0.12
	4	2.3	N.D.
Mean ± S.D.		2.2 ± 0.21	0.10 ± 0.02
126, 25 mg/kg	1	2.4	0.08
	2	2.2	0.05
	3	2.1	0.16
	4	2.2	0.05
Mean ± S.D.		2.2 ± 0.13	0.09 ± 0.05
Corn oil	1	1.7	0.13
	2	1.7	0.09
	3	1.9	0.08
	4	2.1	0.08
	5	1.9	0.08
	6	1.7	0.08
	7	2.4	0.08
Mean ± S.D.		1.9 ± 0.26	0.09 ± 0.02

a - $p < 0.05$, b - $p < 0.01$, c - $p < 0.001$

5. SUMMARY

The results of this research show that Aroclor 1254 caused accumulation of hepatic and renal porphyrins in Japanese quail both as a result of chronic moderate dosing and from single high doses. Aroclor 1254 also increased the activity of both P-450-PB and P-450-MC type polysubstrate monooxygenases. Chronic dosing with PCB congener # 105 caused PSMO induction and porphyrin accumulation that was similar to that caused by Aroclor 1254. Analysis by GC/MS showed that the batch of PCB 105 contained no detectable PCDDs or PCDFs. Congener # 126 caused only slight porphyrin accumulation and minimal PSMO induction, yet appeared to cause thymic atrophy as a result of chronic dosing. The relative toxicity of these two PCB congeners was different in quail than reported for rats. Chronic dosing with PCB 153 caused only minimal induction of MFOs and slight porphyrin accumulation and no other effects.

A single high dose (250 mg/kg) of congener # 77 caused no effects other than slight EROD induction. PCB 77 was not soluble in a range of organic solvents at body temperature and was given as a suspension, which may have affected its uptake by the quail. The same dose of # 153 caused maximal PSMO induction and some porphyrin accumulation as well as liver enlargement. The batch of PCB 153 was found to contain traces of PCDD and PCDF contaminants, which may account for the effects of this compound on both PSMO induction and porphyrin accumulation. A single dose (25 mg/kg) of # 126 caused extreme accumulation of highly carboxylated porphyrins in liver and kidney as well as induction of PSMO activity.

Single high doses of both Aroclor 1254 and individual congeners

caused considerable variation in liver and kidney HCP levels among individual quail. At doses of Aroclor 1254 greater than 100 mg/kg, HCP levels were higher in quail kidney than liver. EROD activity was consistently higher and was increased to a greater degree in liver than kidney. APND activity was consistently higher in liver than in kidney; however, the degree of induction was comparable between the two tissues.

In general, the research calls attention to the need for further work on comparative toxicology of PCBs and other PHAHs in birds. In particular, more studies such as those of Brunstrom & Andersson (1988) are needed to compare the toxicity of the various PHAHs in avian models. More research is also required to characterize the nature of the avian PSMO detoxification systems.

REFERENCES

Anonymous. 1966. Report of a new chemical hazard. New Sci., 32: 621.

Addison, R.F., Brodie, P.F. & Zinck, M.E. 1984. DDT has declined more than PCBs in eastern Canadian seals during the 1970s. Environ. Sci. & Technol., 18: 935-7.

Ballschmiter, K. & Zell, M. 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary chromatography. Fres. Z. Anal. Chem., 302: 20-31.

Ballschmiter, K.H., H. Buchert & S. Bihler. 1981. Baseline studies of global pollution. Fres. Z. Anal. Chem., 306: 323.

Bien, E., Skorka, G., Buntrock, P. & Weubauer, J. 1981. Hepatotoxicological studies of pyrazolone derivatives on rats. 1. The study of aminophenazone. Pharmazie., 36: 492-500.

Bonkovsky, H.L., Sinclair, P.R., Bement, W.J., Lambrecht, R.W. & Sinclair, J.F. 1987. Role of cytochrome P-450 in porphyria caused by halogenated aromatic compounds. In: Silbergeld, E.K. & Fowler, B.A. (eds.) Mechanisms of Chemical-induced Porphyrinopathies. Annals New York Academy of Sciences, 514: 96-112.

Bowes, G.W., Mulvihill, M.J., Simoneit, B.R.T, Burlingame, A.L. & Risebrough, R.W. 1975. Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. Nature, 256, 305-307.

Brown, J.F., Bedard, D.L., Brennan, M.J., Carnahan, J.C., Feng, H. & Wagner, R.E. 1987. Polychlorinated biphenyl dechlorination in aquatic sediments. Science, 236, 709-712.

Brunstrom, B. & Andersson, L. 1988. Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) in chick embryos. Arch. Toxicol., 62, 263-266.

Buckpitt, A.R. & Boyd, M.R. 1982. Metabolic activation of 4-ipomeanol by avian tissue microsomes. Toxicol. Appl. Pharmacol., 43: 507-518.

Bunyan, P.J. & Page, J.M.J. 1978. Polychlorinated biphenyls. The effect of structure on the induction of quail hepatic microsomal enzymes. Toxicol. Appl. Pharmacol., 43: 507-518.

Bursian, S.J., Polin, D. & Olson, B.A. 1983. Microsomal enzyme induction, egg production, and reproduction in three lines of Japanese quail fed polybrominated biphenyls., J. Toxicol. Environ. Health, 12: 291-307.

Cantoni, L., Salmona, M. & Rizzardini, M. 1981. Porphyrinogenic effect of chronic treatment of with 2,3,7,8-tetrachlorodibenzo-p-dioxin in female rats: dose-effect relationships following urinary excretion of porphyrins. Toxicol. Appl. Pharmacol., 57: 156-163.

Cantoni, L., Rizzardini, M., Graziani, A., Carugo, C. & Garattini, S. 1987. Effects of chlorinated organics on intermediates in the heme pathway and on uroporphyrinogen decarboxylase. In: Silbergeld, E.K. & Fowler, B.A. (eds.) Mechanisms of Chemical-induced Porphyrinopathies. Annals New York Academy of Sciences, 514: 128-140

Carpenter, H.M., Williams, D.E. & Buhler, D.R. 1985. A comparison of the effects of hexachlorobenzene, b-naphthoflavone, and phenobarbital on cytochrome P-450 and mixed-function oxidases in Japanese quail. J. Toxicol. Environ. Health, 15: 93-108.

Cecil, H.C., Harris, S.J. & Bitman, J. 1975. Effects of polychlorinated biphenyls and terphenyls and polybrominated biphenyls on pentobarbital sleeping times of Japanese quail. Archiv. Environ. Contam. Toxicol.1, 3: 183-192.

Debets, F.M.H., W.J.H.M.B. Hamers & J.J.T.W.A. Strik. 1980. Metabolism as a prerequisite for the porphyrinogenic action of polyhalogenated aromatics, with special reference to hexachlorobenzene and polybrominated biphenyls (Firemaster BP-6). Int. J. Biochem., 12: 1019-1025.

Environment Canada. 1985. National inventory of fluids containing polychlorinated biphenyls (PCBs). EPS 5/HA/1. Ottawa, Canada. 17 pp.

Fox, G.A., Kennedy, S.W., Norstrom, R.J. & Wigfield, D.C. 1988. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. Environ. Toxicol. and Chem., 7: 831-839.

Friend, M. & Trainer, D.O. 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science, 170: 1314.

Goldstein, J.A., Hickman, P. & Jue, D.L. 1974. Experimental hepatic porphyria induced by polychlorinated biphenyls. Toxicol. Appl. Pharmacol., 27: 437-448.

Goldstein, J.A., J.D. McKinney, G.W. Lucier, P. Hickman, H. Bergman, J.A. Moore. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. Toxicol. & Appl. Pharmacol., 36: 81-92.

Hansen, L.G. 1987. Environmental toxicology of polychlorinated biphenyls. In: Safe, S. (ed.), Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology, Springer-Verlag, Berlin, pp. 15-48.

Heath, R.G., J.W. Spann, E.F. Hill & Kreitzer, J.F. 1972. Comparative dietary toxicities of pesticides to birds. U.S. Fish & Wildl. Serv. Spec. Sci. Rep. Wildl., No. 151.

Henry, R.J., Cannon, D.C. & Winkelman, J.W. 1974. Clinical Chemistry, Principles and Techniques, Harper & Row, 2nd edition.

Hernberg, S., Nikkanen, J., Mellin, G. & Lilius, H. 1970. Alpha-aminolevulinic acid dehydratase as a measure of lead exposure. Archiv. Environ. Health, 23: 440-45.

Hoffman, D.J., Rattner, B.A., Sileo, L., Docherty, D. & Kubiak, T.J. 1987. Embryotoxicity, teratogenicity and aryl hydrocarbon hydroxylase activity in Forster's Terns on Green Bay, Lake Michigan. Environ. Res., 42: 176-184.

Hudson, R.H., Tucker, R.K. & Haegele, M.A. 1984. Handbook of toxicity of pesticides to wildlife, second edition. U.S. Fish Wildl. Serv., Resour. Publ., 154, 90 pp.

Ionnnides, C. & Parke, D.V. 1987. The cytochromes P-448 - a unique family of enzymes involved in chemical toxicity and carcinogenesis. Biochem. Pharmacol., 36: 4197-4207.

Jones, D., Sawyer, T.W., Rosanoff, K. & Safe, S. 1985. Effects of three commercial PCBs and selected congeners in the Avian. Toxicologist, 5:35.

Kannan, N., Tanabe, S. & Tatsukawa, R. 1988. Toxic potential of non-ortho and mono-ortho coplanar PCBs in commercial PCB preparations: "2,3,7,8-T4CDD toxicity equivalence factors approach". Bull. Environ. Contam. Toxicol., 41:267-76.

Kennedy, S.W., D.C. Wigfield & G.A. Fox. 1986a. Tissue porphyrin pattern determination by high-speed high performance liquid chromatography. Anal. Biochem., 157: 1-7.

Kennedy, S.W., Wigfield, D.C. & Fox, G.A. 1986b. The delay in polyhalogenated aromatic hydrocarbon-induced porphyria: mechanistic reality or methodological artefact? Toxicol. Lett., 31:235-41.

Kennedy, S.W. 1988. Studies on Porphyria as an indicator of polyhalogenated aromatic hydrocarbon exposure. Ph.D. thesis. Carleton University, Ottawa. 332 pp.

Kimbrough, R.D. 1974. The toxicity of polychlorinated polycyclic conmpounds and related compounds. Crit. Rev. Toxicol. 2: 445.

Krieger, R.L. & Wilkinson, C.F. 1969. Microsomal mixed function oxidases in insects. 1. Localisation and properties of an enzyme system affecting aldrin epoxidation in the Southern Army Worm (Prodenia eridania). Biochem. Pharmac. 18: 1403-15.

Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E. & Erdman, T.C. 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan - 1983. Archiv. Environ. Contam. Toxicol., 18: in press.

Lambrecht, R.W., Sinclair, P.R., Bement, W.J. & Sinclair, J.F. 1988. Uroporphyrin accumulation in cultured chick embryo hepatocytes: comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol., 96: 507-16.

Leece, B., Denomme, M.A., Towner, R. & Angela Li, S.M. 1985. Polychlorinated biphenyls: correlation between *in vivo* and *in vitro* quantitative structure-activity relationships (QSARs). Toxicol. Environ. Health., 16: 379-388.

Lockwood, G.F. & Houston, J.B. 1979. Aminopyrene disposition in rats. J. Pharm. Pharmac., 31, 787-88.

Macdonald, T.L. 1984. Chemical mechanisms of halocarbon metabolims. CRC Crit. Rev. Toxicol. 11: 85-119.

McConnell, E.E. 1980. Acute and chronic toxicity, carcinogenesis, reproduction teratogenesis and mutagenesis in animals. In: Kimbrough, R.D. (ed.) Halogenated Biphenyls, Terphenyls, Napthalenes, Dibenzodioxins and Related Compounds . Elcevier/North Holland. Amsterdam.

Marks, G.R. 1985. Exposure to toxic agents: the heme biosynthetic pathway and hemoproteins as indicator. CRC Crit. Rev. Toxicol. , 15:151-178.

Meyer, U.A. & Schmid, R. 1973. The Porphyrias. In: Standburg, J.B. & Wyngaarden, J.B. (eds.). The Metabolic Basis of Inherited Disease. McGraw-Hill, New York, pp. 1166-1220.

Mineau, P. G.A. Fox, R.J. Norstrom, D.V. Weseloh, D.J. Hallett & J.A. Ellenton. 1984. Using the herring gull to monitor levels and effects of organochlorine contamination in the Canadian Great Lakes. In: Nriagu, J.O.& Simons, M.S. (eds.) Toxic Contaminants in the Great Lakes. Wiley. Toronto, pp. 425-452.

Miranda, C.L., Henderson, M.C., Wang, J.-L., Nakaue, H.S. & Buhler, D.R. 1986. Induction of acute renal porphyria in Japanese quail by Aroclor 1254. Biochem. Pharmacol. 35: 3637-9.

Miranda, C.L., Henderson, M.C. & Wang, J.-L. 1987. Effects of polychlorinated biphenyls on porphyrin synthesis and cytochrome P-450-dependent monoxygenases in small intestine and liver of Japanese quail. J. Toxicol. Environ. Health, 20: 27-35.

Mullin, M.D., C.M. Pochini, S. McCrindle, M. Romkes, and S.H. Safe. 1984. High resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners. Environ. Sci. Technol., 18: 458-476.

Neal, G.E., Judah, D.J. & Green, J.A. 1986. The *in vitro* metabolism of aflatoxin B_1 catalyzed by hepatic microsomes isolated from control or 3-methylcholanthrene-stimulated rats and quail. Toxicol. Appl. Pharmacol., 82, 454-460.

Nebert, D.W. & H.V. Gelboin. 1968. Substrate inducible microsomal aryl hydroxylase in mammalian cells. 1. Assay and properties of induced enzyme. J. Biol. Chem., 243: 6242-49.

Nebert, D.W. & Gonzalez. 1985. Cytochromi P-450 gene expression and regulation , Trends Pharmac. Sci., 6: 160.

Nebert, D.W., H.J. Eisen, M. Negishi, M.A. Lang, L.M. Hjelmeland. 1981. Genetic mechanisms controlling the induction of polysubstrate monooxygenase activites. Ann. Rev. Pharmacol. Toxicol., 21: 431-62.

Nikolaides, E., Brunstrom, B. & Dencker, L. 1988. Effects of the TCDD congeners 3,3',4,4'-tetrachlorobiphenyl and 3,3',4,4'-tetrachloroazoxybenzene on lymphoid development in the bursa of Fabricus of the chick embryo. Toxicol. Appl. Pharmacol., 92: 315-323.

Norstrom, R.J. 1988. Bioaccumulation of polychlorinated biphenyls in Canadian wildlife. In: Crine, J.-P. (ed.), Hazards, decontamination and replacement of PCBs, Plenum Publishing Corp., N..Y. (in press).

Norstrom, R.J., Simon, M. & Mulvihill, M.J. 1986. A gel-permeation/column chromatography cleanup method for the determination of CDDs in animal tissue. Internat. J. Environ. Anal. Chem., 23: 267-287.

Olson, M. & Reutergardh, L. 1986. DDT and PCB pollution trends in the Swedish aquatic environment. Ambio, 15: 103-9.

Ontario Ministry of Environment. 1985. Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Scientific criteria document for standard development, No. 4-84.

Parkinson, A. & Safe, S. 1981. Aryl hydrocarbon hydroxylase induction and its relationship to the toxicity of halogenated hydrocarbons. Toxicol. Environ. Chem. Rev., 4: 1.

Parkinson, A., Copp, L. & Safe, S. 1980. The utility of the microsomal 4-chlorobiphenyl hydroxylase enzyme assay in distinguishing between phenobarbitone and 3-methylcholanthrene-induced microsomal monooxygenases. Anal. Biochem., 105: 65-73.

Parkinson,, A., Cockerline, R. & Safe, S. 1980. Polychlorinated biphenyl isomers and congeners as inducers of both 3-methylcholanthrene and phenobarbitone-type microsomal enzyme activity. Chem. Biol. Interact., 29: 259.

Passivirta, J. & Linko, R. 1980. Environmental toxins in Finnish wildlife. A study of trends of residue contents in fish during 1973-1978. Chemosphere, 9: 643.

Pearce, P.A., Elliott, J.E., Peakall, D.B. & Norstrom, R.J. Organochlorine contaminants in eggs of seabirds in the northwest Atlantic, 1968-1984. Environ. Pollut., 56: in press.

Peterson, G.L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem., 83: 346-56.

Platanow, N.S. & L.H. Karsted. (1973). Dietary effects of polychlorinated biphenyls on mink. Can. J. Comp. Med. 37: 391-400.

Platanow, N.S. & Reinhar, B.S. 1973. The effects of polychlorinated biphenyls (Aroclor 1254) on chicken egg production, fertility and hatchability. Can. J. Compar. Med., 37: 341-346.

Poland, A., J. Knutson & E. Glover. 1983. A consideration of the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons. In: Tucker, R.E. Young, A.L. & Gray, A.P. (eds.). Human and Environmental risks of Chlorinated Dioxins and Related Compounds. Plenum Press. N.Y., pp. 539-562.

Pyykko, K. 1983. Characterization and stability of rat liver microsomes isolated by a rapid gel filtration method. Acta Pharmacol. Toxicol., 52: 39-46.

Rifkind, A.B., Troeger, M. & Muschick, H. 1982. Kinetic evidence for heterogeneous responsiveness of mixed function oxidase isozymes to inhibition and induction by allylisopropylacetamide in chick embryo liver. J. Biol. Chem., 257: 11717-11727.

Rifkind, A.B., Firpo, A. & Alonso, D.R. 1984. Coordinate induction of cytochrome P-448 mediated mixed function oxidases and histopathologic changes produced acutely in chick embryo liver by polychlorinated biphenyl congeners. Toxicol. Appl. Pharmacol., 72: 343-54.

Rifkind, A.B., Sassa, S., Reyes, J. & Muschick, H. 1985. Polychlorinated aromatic hydrocarbon lethality, mixed function oxidase induction, and uroporphyrinogen decarboxylase inhibition in the chick embryo: dissociation of dose-response relationships. Toxicol. Appl. Pharmacol., 78: 268-79.

Rinzky, A. & Perry. A.S. 1983. Postnatal development of the mixed function oxidase system in nestling barn owls and baby chicks and induction of this system in the mature forms by Aroclor 1254. Comp. Biochem. Physiol., 75C: 51-55.

Risebrough, R.W., P.Rieche, S.G. Herman, D.B. Peakall & M.N. Kirven. 1968. Polychlorinated biphenyls in the global ecoystem. Nature, 220: 1098.

Riviere, J.L., G. Grolleau & J. Bach. 1985. Hepatic biotransformation in the buzzard (Buteo buteo) and the Japanese quail (Coturnix coturnix): effect of PCBs. Comp. Biochem. Physiol., 82C: 439-43.

Safe, S. 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanisms of action. CRC Crit. Rev. Toxicol., 13: 319-369.

Safe, S (ed.). 1987. Polychlorinated biphenyls (PCBs): mammalian and environmental toxicology, Springer-Verlag, New York, pp.

Sano, S., Kawanishi, S. & Seki, Y. 1985. Toxicity of polychlorinated biphenyl with special reference to porphyrin metabolism. Environ. Health Perspect., 59: 137-143.

Sassa, S., Sugita, O., Ohnuma, N., Imajo, S., Okumura, T., Noguchi, T. & Kappas, A. 1986. Studies of the influence of chloro-substituent sites and conformational enery in polychlorinated biphenyls on uroporphyrin formation in chick-embryo liver cell cultures. Biochem. J., 235: 21-296.

Sawyer, T.W. & Safe, S. 1982. PCB isomers and congeners: induction of aryl hydrocarbon hydroxylase and ethoxyresorufin o-deethylase enzyme activities in rat hepatoma cells. Toxicol. Lett., 13: 87-94.

Sawyer, T.W., Vatcher, A.D. & Safe, S. 1984. Comparative aryl hydrocarbon hydroxylase induction activities of commercial PCBs in Wistar rats and rat hepatoma H-4-II E cells in culture. Chemosphere, 13: 695-701.

Sifri, M., Sell, J.L. & Davison, K.L. 1975. Comparative effects of p,p'-DDT and pentobarbital on hepatic microsomal enzymes in young quail, chicks and ducklings. Comp. Biochem. Physiol., 51B, 213-219.

Sinclair, P.R., W.J. Bement, H.L. Bonkovsky, R.W. Lambrecht, J.E. Frezza, J.F. Sinclair, A.J. Urquhart & G.H. Elder. 1986. Uroporphyrin accumulation produced by halogenated biphenyls in chick-embryo hepatocytes. Biochem. J. 237: 63-71.

Sinclair, P.R., Lambrecht, R. & Sinclair, J. (1987). Evidence for cytochrome P450-mediated oxidation of uroporphyrinogen by cell-free liver extracts from chick embryos treated with 3-methylchlolanthrene. Biochem. Biophys. Res. Commun., 146:1324-29.

Stauffer, C.E. 1975. A linear standard curve for the folin lowry determination of protein. Anal.Biochem., 69, 649-51.

Stonard, M.D. & Greig, J.B. 1976. Different patterns of hepatic microsomal enzyme activity produced by administration of pure hexachlorobiphenyl isomers and hexachlorobenzene. Chem. Biol. Interactions, 15, 365-79.

Stout, V. F. 1987. What is happending to PCBs? Elements of environmental monitoring as illustrated by an analysis of PCB trends in terrestial and aquatic organisms. In: Waid, J.S. (ed.) PCBs and the Environment, CRC Press, Boca Raton, Florida, pp. 163-206.

Swain, M.G., S.B. Follows & G.S. Marks. 1983 Inhibition of uroporphyrinogen decarboxylase by 3,3',4,4'-tetrachlorobiphenyl in chick embryo liver cell culture. Can. J. Physiol. Pharmacol., 61: 105.

Tanabe, S. (1988). PCB problems in the future: foresight from current knowledge. Environ. Pollut., 50: 5-28.

Tanabe, S., Kannan, N., Subramanian, An., Watanabe, S., Tatsukawa, R. (1987). Highly toxic coplanar PCBs: occurence, source, persistency and toxic implication. Environ. Pollut., 47: 147-163.

Waid, J.S. 1987. PCBs and the Environment, CRC Press, Baton Roca, Florida, 228 pp.

Vos, J.G. & Koeman, J.H. 1970. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. Toxicol. Appl. Pharmacol., 17: 656-668.

Vos, J.G., Strik, J.J.T.W.A., van Holsteyn, W.M. & Pennings, J.H. 1971. Polychlorinated biphenyls as inducers of hepatic porphyria in Japanese quail, with special reference to alpha-aminolevulinic acid synthetase activity, fluorescence and residues in the liver. Toxicol. Appl. Pharmacol., 20: 232-240.

Yamamoto, H., Yoshimura, H. Funita, M. & Yamamoto, T. 1976. Metabolic and toxicologic evaluation of 2,3,4,3',4'-pentachlorobiphenyl in rats and mice. Chem. Pharm. Bull., 24: 2168.

Zelt, D.T. 1980. Interactions of chemicals with the liver heme biosynthetic pathway with special reference to polychlorinated biphenyls. M.Sc. thesis, Queens Univ. Kingston, Ontario. 143 pp.

APPENDIX A

NUMBERING SYSTEM FOR PCB CONGENERS

The polychlorinated biphenyl congeners listed below are arranged in sequence according to IUPAC rules for substituent characterization of biphenyls and then numbered in arithmetic progression (Ballschmiter & Zell, 1980).

BZ#	Structure
—	Biphenyl
1	2-Chlorobiphenyl
2	3-Chlorobiphenyl
3	4-Chlorobiphenyl
4	2,2'-Dichlorobiphenyl
5	2,3-Dichlorobiphenyl
6	2,3'-Dichlorobiphenyl
7	2,4-Dichlorobiphenyl
8	2,4'-Dichlorobiphenyl
9	2,5-Dichlorobiphenyl
10	2,6-Dichlorobiphenyl
11	3,3'-Dichlorobiphenyl
12	3,4-Dichlorobiphenyl
13	3,4'-Dichlorobiphenyl
14	3,5-Dichlorobiphenyl
15	4,4'-Dichlorobiphenyl
16	2,2',3-Trichlorobiphenyl
17	2,2',4-Trichlorobiphenyl
18	2,2',5-Trichlorobiphenyl
19	2,2',6-Trichlorobiphenyl
20	2,3,3'-Trichlorobiphenyl
21	2,3,4-Trichlorobiphenyl
22	2,3,4'-Trichlorobiphenyl
23	2,3,5-Trichlorobiphenyl
24	2,3,6-Trichlorobiphenyl
25	2,3',4-Trichlorobiphenyl
26	2,3',5-Trichlorobiphenyl
27	2,3',6-Trichlorobiphenyl
28	2,4,4'-Trichlorobiphenyl
29	2,4,5-Trichlorobiphenyl
30	2,4,6-Trichlorobiphenyl
31	2,4',5-Trichlorobiphenyl
32	2,4',6-Trichlorobiphenyl
33	2',3,4-Trichlorobiphenyl
34	2',3,5-Trichlorobiphenyl
35	3,3',4-Trichlorobiphenyl
36	3,3',5-Trichlorobiphenyl
37	3,4,4'-Trichlorobiphenyl
38	3,4,5-Trichlorobiphenyl
39	3,4',5-Trichlorobiphenyl
40	2,2',3,3'-Tetrachlorobiphenyl
41	2,2',3,4-Tetrachlorobiphenyl
42	2,2',3,4'-Tetrachlorobiphenyl
43	2,2',3,5-Tetrachlorobiphenyl
44	2,2',3,5'-Tetrachlorobiphenyl
45	2,2',3,6-Tetrachlorobiphenyl
46	2,2',3,6'-Tetrachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl
48	2,2',4,5-Tetrachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl
50	2,2',4,6-Tetrachlorobiphenyl
51	2,2',4,6'-Tetrachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl
53	2,2',5,6'-Tetrachlorobiphenyl
54	2,2',6,6'-Tetrachlorobiphenyl
55	2,3,3',4-Tetrachlorobiphenyl
56	2,3,3',4'-Tetrachlorobiphenyl
57	2,3,3',5-Tetrachlorobiphenyl
58	2,3,3',5'-Tetrachlorobiphenyl
59	2,3,3',6-Tetrachlorobiphenyl
60	2,3,4,4'-Tetrachlorobiphenyl
61	2,3,4,5-Tetrachlorobiphenyl
62	2,3,4,6-Tetrachlorobiphenyl
63	2,3,4',5-Tetrachlorobiphenyl
64	2,3,4',6-Tetrachlorobiphenyl
65	2,3,5,6-Tetrachlorobiphenyl
66	2,3',4,4'-Tetrachlorobiphenyl
67	2,3',4,5-Tetrachlorobiphenyl
68	2,3',4,5'-Tetrachlorobiphenyl
69	2,3',4,6-Tetrachlorobiphenyl
70	2,3',4',5-Tetrachlorobiphenyl
71	2,3',4',6-Tetrachlorobiphenyl
72	2,3',5,5'-Tetrachlorobiphenyl
73	2,3',5',6-Tetrachlorobiphenyl
74	2,4,4',5-Tetrachlorobiphenyl
75	2,4,4',6-Tetrachlorobiphenyl
76	2',3,4,5-Tetrachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl
78	3,3',4,5-Tetrachlorobiphenyl
79	3,3',4,5'-Tetrachlorobiphenyl
80	3,3',5,5'-Tetrachlorobiphenyl
81	3,4,4',5-Tetrachlorobiphenyl
82	2,2',3,3',4-Pentachlorobiphenyl
83	2,2',3,3',5-Pentachlorobiphenyl
84	2,2',3,3',6-Pentachlorobiphenyl
85	2,2',3,4,4'-Pentachlorobiphenyl
86	2,2',3,4,5-Pentachlorobiphenyl
87	2,2',3,4,5'-Pentachlorobiphenyl
88	2,2',3,4,6-Pentachlorobiphenyl
89	2,2',3,4,6'-Pentachlorobiphenyl
90	2,2',3,4',5-Pentachlorobiphenyl
91	2,2',3,4',6-Pentachlorobiphenyl
92	2,2',3,5,5'-Pentachlorobiphenyl
93	2,2',3,5,6-Pentachlorobiphenyl
94	2,2',3,5,6'-Pentachlorobiphenyl
95	2,2',3,5',6-Pentachlorobiphenyl
96	2,2',3,6,6'-Pentachlorobiphenyl
97	2,2',3',4,5-Pentachlorobiphenyl
98	2,2',3',4,6-Pentachlorobiphenyl
99	2,2',4,4',5-Pentachlorobiphenyl
100	2,2',4,4',6-Pentachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
102	2,2',4,5,6'-Pentachlorobiphenyl
103	2,2',4,5',6-Pentachlorobiphenyl
104	2,2',4,6,6'-Pentachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
106	2,3,3',4,5-Pentachlorobiphenyl
107	2,3,3',4',5-Pentachlorobiphenyl
108	2,3,3',4,5'-Pentachlorobiphenyl
109	2,3,3',4,6-Pentachlorobiphenyl
110	2,3,3',4',6-Pentachlorobiphenyl
111	2,3,3',5,5'-Pentachlorobiphenyl
112	2,3,3',5,6-Pentachlorobiphenyl
113	2,3,3',5',6-Pentachlorobiphenyl
114	2,3,4,4',5-Pentachlorobiphenyl
115	2,3,4,4',6-Pentachlorobiphenyl
116	2,3,4,5,6-Pentachlorobiphenyl
117	2,3,4',5,6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
119	2,3',4,4',6-Pentachlorobiphenyl
120	2,3',4,5,5'-Pentachlorobiphenyl
121	2,3',4,5',6-Pentachlorobiphenyl
122	2',3,3',4,5-Pentachlorobiphenyl
123	2',3,4,4',5-Pentachlorobiphenyl
124	2',3,4,5,5'-Pentachlorobiphenyl
125	2',3,4,5,6'-Pentachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
127	3,3',4,5,5'-Pentachlorobiphenyl
128	2,2',3,3',4,4'-Hexachlorobiphenyl
129	2,2',3,3',4,5-Hexachlorobiphenyl
130	2,2',3,3',4,5'-Hexachlorobiphenyl
131	2,2',3,3',4,6-Hexachlorobiphenyl
132	2,2',3,3',4,6'-Hexachlorobiphenyl
133	2,2',3,3',5,5'-Hexachlorobiphenyl
134	2,2',3,3',5,6-Hexachlorobiphenyl
135	2,2',3,3',5,6'-Hexachlorobiphenyl
136	2,2',3,3',6,6'-Hexachlorobiphenyl
137	2,2',3,4,4',5-Hexachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
139	2,2',3,4,4',6-Hexachlorobiphenyl
140	2,2',3,4,4',6'-Hexachlorobiphenyl
141	2,2',3,4,5,5'-Hexachlorobiphenyl
142	2,2',3,4,5,6-Hexachlorobiphenyl
143	2,2',3,4,5,6'-Hexachlorobiphenyl
144	2,2',3,4,5',6-Hexachlorobiphenyl
145	2,2',3,4,6,6'-Hexachlorobiphenyl
146	2,2',3,4',5,5'-Hexachlorobiphenyl
147	2,2',3,4',5,6-Hexachlorobiphenyl
148	2,2',3,4',5,6'-Hexachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl
150	2,2',3,4',6,6'-Hexachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl
152	2,2',3,5,6,6'-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
154	2,2',4,4',5,6'-Hexachlorobiphenyl
155	2,2',4,4',6,6'-Hexachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl
159	2,3,3',4,5,5'-Hexachlorobiphenyl
160	2,3,3',4,5,6-Hexachlorobiphenyl
161	2,3,3',4,5',6-Hexachlorobiphenyl
162	2,3,3',4',5,5'-Hexachlorobiphenyl
163	2,3,3',4',5,6-Hexachlorobiphenyl
164	2,3,3',4',5',6-Hexachlorobiphenyl
165	2,3,3',5,5',6-Hexachlorobiphenyl
166	2,3,4,4',5,6-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
168	2,3',4,4',5',6-Hexachlorobiphenyl
169	3,3',4,4',5,5'-Hexachlorobiphenyl
170	2,2',3,3',4,4',5-Heptachlorobiphenyl

BZ#	Structure
171	2,2',3,3',4,4',6-Heptachlorobiphenyl
172	2,2',3,3',4,5,5'-Heptachlorobiphenyl
173	2,2',3,3',4,5,6-Heptachlorobiphenyl
174	2,2',3,3',4,5 ,6'-Heptachlorobiphenyl
175	2,2',3,3',4,5',6-Heptachlorobiphenyl
176	2,2',3,3',4,6,6'-Heptachlorobiphenyl
177	2,2',3,3',4',5,6-Heptachlorobiphenyl
178	2,2',3,3',5,5',6-Heptachlorobiphenyl
179	2,2',3,3',5,6,6'-Heptachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
181	2,2',3,4,4',5,6-Heptachlorobiphenyl
182	2,2',3,4,4',5,6'-Heptachlorobiphenyl
183	2,2',3,4,4',5',6-Heptachlorobiphenyl

BZ#	Structure
184	2,2',3,4,4',6,6'-Heptachlorobiphenyl
185	2,2',3,4,5,5',6-Heptachlorobiphenyl
186	2,2',3,4,5,6,6'-Heptachlorobiphenyl
187	2,2',3,4',5,5',6-Heptachlorobiphenyl
188	2,2',3,4',5,6,6'-Heptachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
190	2,3,3',4,4',5,6-Heptachlorobiphenyl
191	2,3,3',4,4',5',6-Heptachlorobiphenyl
192	2,3,3',4,5,5',6-Heptachlorobiphenyl
193	2,3,3',4',5,5',6-Heptachlorobiphenyl
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
196	2,2',3,3',4,4',5',6-Octachlorobiphenyl

BZ#	Structure
197	2,2',3,3',4,4',6,6'-Octachlorobiphenyl
198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
199	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
200	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
201	2,2',3,3',4',5,5',6-Octachlorobiphenyl
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl
203	2,2',3,4,4',5,5',6-Octachlorobiphenyl
204	2,2',3,4,4',5,6,6'-Octachlorobiphenyl
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl
208	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl
209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl

APPENDIX B

PCDD AND PCDF CONTAMINATION OF PCB CONGENERS

Due to concerns over the potent EROD induction caused by PCB 153 in the study in Chapter 3, an effort was made to analyze it for possible contamination. The analyses were conducted by Dr. Ross Norstrom of CWS and the technique used is outlined in the Methods section. The results are on page 83.

As a result of the findings for # 153, # 105 was also analyzed. As # 105 has been shown to induce a P-450-MC type response in rats, it was important to determine whether trace contaminants were contributing to effects seen with this compound. No PCDD or PCDF contaminants were found in the batch of # 105.

PCB # 77 was not analyzed as it caused minimal EROD induction. PCB # 126 was not analyzed firstly because it is a very potent P-450-MC type inducer, thus trace PCDD and PCDF contamination would probably make a negligible contribution to its toxicity. Secondly, there was concern with both coplanar congeners for contamination of analytical equipment by the relatively large amounts of the congeners needed for analysis.