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THF EFFECTS OF DIETARY AROCLOR 1254
ON TISSUE AND WHOLE-BODY RESIDUES AND
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By

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ABSTRACT

THE EFFECTS OF DIETARY AROCLOR 1254 ON TISSUE AND WHOLE-BODY RESIDUES AND 7-ETHOXYRESORUFIN ACTIVITY IN AMERICAN BUDGERIGARS

By

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American budgerigars (budgies) were fed dietary Aroclor 1254 at concentrations of 0, 12.5, 25, 50, or 100 ppm for 28 days. Body weight, feed consumption, and mortality were measured on 50 budgies. The skin contained the highest concentration of Aroclor 1254 (774 ppm) followed by the liver (144 ppm), kidney (59 ppm), muscle (6.3 ppm) and plasma (4.1 ppm). The liver, kidney and brain were measured as a percent body weight. Relative liver weights averaged 1.79% where as the treated groups ranged from 2.0% to 3.0%. The kidney and brain weights were not affected by Aroclor 1254. Microsomal protein concentration of the liver and microsomal cytochrome P_{450} dependent EROD activity were measured in a second experiment identical to the first. Microsomal protein concentration in the control group contained 1.07 mg/g liver where as the treated groups contained an average of 2.61 mg/g liver. 7-ethoxyresorufin activity was maximally induced at the lowest dietary Aroclor 1254 concentration, 12.5 ppm (72 mg resorufin/mg protein/minute) as compared to the control 0.29 mg/mg/min.

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INTRODUCTION

In 1881, Schmidt and Schultz described the synthesis of polychlorinated biphenyls (PCBs) (Cairnes et al., 1986). The industrial importance of PCBs was not fully realized until 1929. This, in turn, initiated widespread production of PCBs. Industrial chemicals for use as nonflammable oils and heat resistant products opened the door to a series of commercially available raw materials marketed under various trade names (Cairnes et al., 1986). In 1971, Monsanto recognized the environmental problems which had developed because of PCBs and began a program to phase out sales of domestic production while limiting sales to uses in electrical capacitors and transformers (Hess, 1976).

PCBs have been increasingly identified as a significant environmental pollutant (Durfee, 1976). Figure 1 is a schematic diagram of the environmental fate and transport of PCBs from different matrices (Safe et al., 1985). Causes for concern include their environmental persistence, potential for biological magnification, and chronic toxicity (Hess, 1976). The highly lipophilic character of these contaminants contributes significantly to the food chain. Such examples (Cook, 1972) of human exposure to PCBs either directly or indirectly is possible through:

1. The food chain, such as the accidental contamination of rice oil that occurred in Japan.

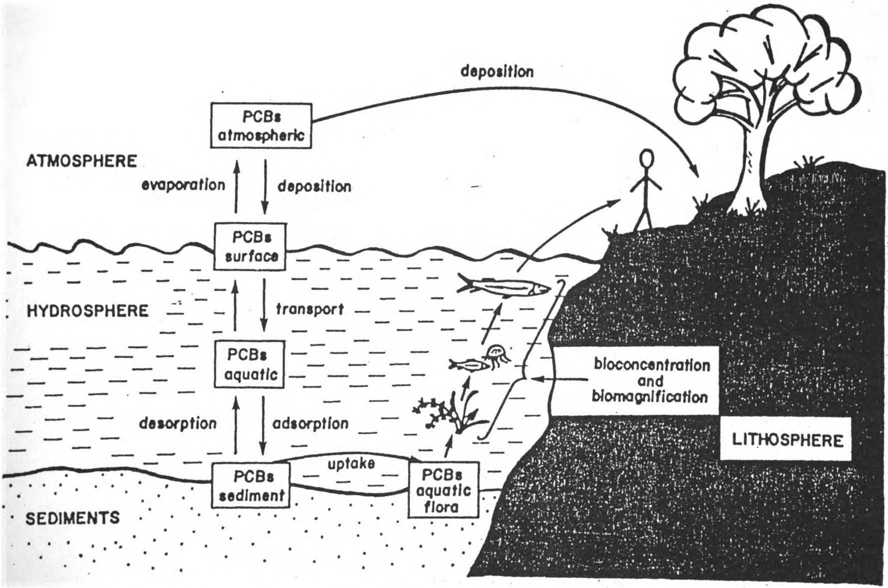

FIGURE 1 A SCHEMATIC DIAGRAM OF THE ENVIRONMENTAL FATE AND TRANSPORT OF PCBs FROM DIFFERENT MATRICES (Safe et al., 1985)

2. Packaging material leaking into and contaminating the food.
3. Contaminated feed is consumed by food producing animals and humans consume by-products from those animals.
4. Aquatic species are exposed by consuming smaller aquatic contamninated life form.

The Food and Drug Administration has set tolerance limits for PCBs in several classes of food (Cairns et al., 1986):

	PCBs (ppm)	
	1973	1979
Milk and dairy products (fat basis)	2.0	1.5
Poultry (fat basis)	5.0	3.0
Eggs	0.5	0.3
Fish and Shellfish (edible portion)	5.0	5.0
Infant and junior foods	0.2	0.2
Feed for food-producing animals	0.5	0.2
Animal feed components including fishmeal and other by-products of marine origin	5.0	2.0
Paper food packaging material intended for or used with human food	10.0	10.0

OBJECTIVES

The objectives of this study are:

1. To determine if Aroclor 1254, fed at high concentrations in the diet, is toxic to the American Budgerigar (Budgie), also referred to as a parakeet.
2. To determine the residues in selected tissues from feeding Aroclor 1254 to the American Budgerigar.
3. To determine the activity of hepatic 7-ethoxyresorufin-O-deethylase (EROD) in budgie livers.

PRODUCTION AND USAGE OF PCBs

Polychlorinated biphenyls (PCBs) are commercial products which are prepared industrially from the iron catalyzed chlorination of biphenyl (Zabik, 1983). Commercial preparations are graded and marketed under various tradenames throughout the world. U.S.A., Great Britain, Germany, France, Japan, Italy, U.S.S.R and Czechoslovakia are countries that manufacture PCBs. A complete list of tradenames and manufacturers is listed in Hutzinger et al. (1974). Monsanto, located in Sauget, Illinois, was the manufacturer of PCBs for the U.S.A. and Great Britain. Information regarding PCBs is most often represented by the Aroclors, therefore this particular brand will serve as a model of various aspects of PCBs in general (Hutzinger et al., 1974). Common Aroclor mixtures include 1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268 and 1016 (Mieure et al., 1976).

Manufacturing PCBs occurs in multiple steps at different plants. The raw material is biphenyl and is manufactured from pure benzene (Durfee, 1976). The first major step in processing PCBs occurs at the Krummich Monsanto plant (Durfee, 1976).

Biphenyl with ferric chloride as the catalyst, and anhydrous chlorine are heated to melting while chlorine gas is introduced into the system and a charge is produced with an electric pump to allow the reaction to proceed. The degree of product chlorination is controlled by the time of chlorine contact. Vapors from the chlorination process are scrubbed and removed to another part of the plant. The crude Aroclor is held at constant temperature and blown dry with air for several hours. A small amount of lime is added to remove any additional HCL or ferric chloride. Finally, the blown air is scrubbed and vented through a demister. Purification of the product is also described in Durfee (1976). During this process of purification, variation occurs due to different chlorine products. For example, the 1016 unit material is processed in a gas fired retort and then transferred to a vacuum distillation to remove the more highly chlorinated compounds. The first cut is recycled back to the retort. A preset overhead temperature must be reached before the product is sent to storage and then shipment. Other Aroclors like 1254 and 1248 are also vacuum distilled. However, these are only distilled once as the condensate is the product. The still bottoms are referred to as Montars and are incinerated at the end of the process.

Monsanto reported domestic sales of PCBs in the U.S. by use and metric tons (Hutzinger et al., 1974). Their production and sales figures for 1960 reveal that over 40 million pounds were produced, of which 86% was domestically sold, and 14% exported. By 1970, sales had doubled to 80 million pounds in the U.S. (Durfee, 1976 and Hess, 1976). There was a large decline in production output after 1970 due to a voluntary restriction of sales by Monsanto (Hutzinger et al., 1974). Environmental contamination was the major concern that initiated actions taken to investigate a potential problem. Durfee (1976) mentioned possibilities of accidental leakage during processing and storage and incorrect disposal practices that lead to environmental contamination. An estimated 750 million pounds of PCBs are still in use today in capicators and transformers (Hutzinger et al., 1985).

A company in Michigan reported in a questionnaire an annual use of 562,000 lbs. of PCBs in the state (250,000 lbs. in precipitator transformers and 312,000 lbs. in capacitors). Annual losses were approximately 300 lbs. of PCB liquids to soils from broken capacitors and 12,000 lbs. from salvage capacitors (Hess, 1976). Disposal of this fluid was through waste haulers who then disposed of it in various ways, such as application on dirt roads for dust control and sale of oils as fuels in low-temperature boilers.

In October 1975, a Michigan power company reported a similar incident (Hess, 1976). A 55 gallon drum containing PCB transformer oil developed a leak from a defective seam. Forty-five gallons of the fluid soaked into the ground, resulting in 100 cubic yards of contaminated soil. This area was removed and disposed of in an approved landfill. This is just one of the many reported accidents representing an example of environmental loss from a so-called "closed-system".

PCBs had numerous uses in hydraulic systems involving extreme pressure and high-temperature conditions (Hess, 1976). Monsanto reported that domestic sales of PCBs for hydraulic uses totaled 8 million lbs. in 1970 (Hutzinger et al., 1974). In another Michigan survey, an industry reported a loss of 30,000 lbs. of PCBs per year in hydraulic systems. The calculated losses included vaporization of 10,000 lbs., 8000 lbs. as disposed of in landfills, 6000 lbs. lost to soils and drainage systems, 5000 lbs. disposed of by waste haulers and the remainder incinerated (Hess, 1976).

The investment casting wax industry has used PCBs, primarily decachlorobiphenyl, for production of low-tolerance quality metal castings. The process involves two steps with possible loss and environmental contamination due to vaporization, cooling water contact, and poor housekeeping practices (Durfee, 1976 and Hess, 1976).

The incidents reported annually are only a fraction of the actual number of environmental PCB contamination, many incidents go unreported.

PHYSICAL AND CHEMICAL PROPERTIES OF PCBs

A basic knowledge of PCB structure and nomenclature is important to understand the diversity of the compound. All Aroclor products consist of a 4-digit number. The first two digits represent the type of molecule. For example, 12 denotes a chlorinated biphenyl molecule with 12 attachment sites, although the first site on each ring is used for the biphenyl bridge leaving 10 possible attachments sites. The second two digits represent the percent of chlorine by weight. For example, Aroclor 1254 contains 54% chlorine by weight. An exception to this rule is Aroclor 1016, which contains 41% chlorine by weight. The chlorine content, molecular weight, and the proportion of the various chlorobiphenyls in four common Aroclors have been reported by Cairns et al. (1986). Aroclor 1254, for instance, contains 54.3% chlorine, consists of an average of 4.96 chlorine atoms per molecule, has an average molecular weight of 326.43, and contains 49% pentachlorobiphenyl, 34% hexachlorobiphenyl, 11% tetrachlorobiphenyl and 6% heptachlorobiphenyl.

Both benzene rings are numbered identically. To distinguish between the two, the ring with the lower number of chlorine atoms attached is usually the primary ring. If both benzene rings have equal number of chlorine atom attachments then the highest numerical position becomes precedent (Mieure et al., 1976; Sawhney, 1986). Examples of PCB structure and nomenclature are shown in Mieure et al. (1976).

Theoretically, there are 209 possible chlorobiphenyl isomers. However, many of these isomers are not likely to occur at significant levels in commercial PCB mixtures (Mieure et al., 1976). Possible chlorine substitutions are shown in Cook (1972).

The physical and chemical properties of PCB isomers and commercial mixtures vary greatly depending on the degree of chlorination and position of chlorine substitution (Cairns et al., 1986; Hutzinger et al., 1974; Mieure et al., 1976). Physical, chemical and electrical properties differ among the different Aroclors. Since Aroclor 1254 was used in this study, the characteristics of this mixture will be mentioned. For properties common to the other Aroclors, refer to Cairns et al. (1986), Hutzinger et al. (1974), and Mieure et al. (1976). Aroclor 1254 is a light yellow viscous liquid which is 54% chlorine by weight. It has a density of 1.53 g/ml and is soluble in water at 0.053 ppm. The biodegradability of Aroclor 1254 is 15% degradation/48 hr cycle. It has a dielectric constant at 1000 cycles of 5.0 @ 25 ^{o}C and 4.3 @ 100 ^{o}C. As a group, the higher the degree of chlorination, the higher the specific gravity, density and distillation range. Water solubility, vaporization rate, biodegradability and dielectric constant appear to decrease with increasing chlorination (Hutzinger et al., 1974).

PCBs are among the most stable organic compounds

(Cairns et al., 1986). The two factors which make them useful in transformers and capacitors are their low dielectric constants and high heat capacity. However, from an environmental point of view, the two most important physical properties are solubility and vapor pressure (Hutzinger et al., 1974). The solubility of PCBs in water and in organic solvents greatly influences their transport and persistence in the environment (Cairns et al., 1986). As previously stated, the solubility of PCBs in water generally decreases with increasing chlorine content. Individual chlorobiphenyls showed a wide range of variation in solubility.

For instance, the solubility of monochlorobiphenyl equals 6 ppm as compared to octachlorobiphenyl which has a water solubility of 0.007 ppm (Cairns et al., 1986). Variation among congeners with the same number of chlorine at different positions was studied by Hutzinger et al. (1974), who reported that solubilities of 2,4-, 2,2'-, and 2,4'-dichlorobiphenyls were 1.40, 1.50, and 1.88 ppm, respectively, whereas 4,4'-dichlorobiphenyl solubility is only 0.08 ppm. PCBs have very low vapor pressures which, as with water solubility, decrease with increasing chlorine content (Cairns et al., 1986). Cairns et al. (1986) reported that those Aroclors consisting of low percent chlorine, such as Aroclor 1221, and containing primarily mono- and dichlorobiphenyls, tended to have vaporization rates which were approximately 200 fold less than Aroclor 1260 which contained penta-, hexa-, hepta- and octachlorobiphenyl.

Sorption reactions of PCBs have received considerable attention. Transport and fate of PCBs in aquatic systems and their partitioning in different compartments of the environment depend greatly on the sorption-desorption rates of individual chlorobiphenyls comprising the mixture (Cairns et al., 1986). Studies were done on the degree of sorption using individual chlorobiphenyls. Different sorbents, such as sand, soil, clay and humic acid were used in the study. The order of sorption of the chlorobiphenyls were hexa-> tetra-> dichlorobiphenyl. They also reported the higher the chlorination, the tighter the PCB compound held to the sorbent surface (Haque and Schmedding, 1976). Aroclor 1254 was used in the study of desorption reactions and the data suggested there was less vapor loss of the higher chlorinated biphenyls (Haque et al., 1974).

Biodegradation of PCBs has been investigated in many studies. In soil plant systems and compiled microorganism sources, results showed biodegradation products of a number of chlorinated biphenyls and commercial PCB preparations (Pal et al., 1980). In addition, stimulation of bacterial growth by 500 ppm of Aroclors 1221, 1242 and 1254 as the carbon and energy sources demonstrated PCB metabolism by bacteria (Wong and Kaiser, 1975).

In 1976, losses of transformer oil occurred in a transformer manufacturing plant in Regina, Saskatchewan, Canada (Roberts et al., 1982). Askarel Inerteen 70-30 was the dielectric fluid in the transformers and manufactured at the Regina plant. This oil contained 70% Aroclor 1254 and 30% chlorobenzenes. The exact date of the losses was uncertain due to slow leakage from an underground pipe which was the cause of the problem. Between 6800 and 21,000 liters of water and oil were collected from many trenches and shallow sumps in the building. Field studies began at the site in 1978 when continued seepage and increasing public and government concerns were rising. Results from the survey showed that large quantities of PCBs were present in three major zones under the building site. The first zone consisted of relatively high levels of PCBs (>500 ppm) detected throughout the Regina clay. Zone two contained residues in excess of 1000 ppm PCBs in a number of shallow areas, and in zone three, there were areas of erosion with low level contamination (<500 pmm) (Roberts et al., 1982). Atwater (1984), did a follow up study on the site and concluded that 1). surface PCBs were adsorbed on the soil; 2). PCBs migrated laterally in the granular fill under the manufacturing plant; and 3). PCBs migrated vertically and horizontally through fractures in the Regina clay.

METABOLISM AND PHARMACOKINETICS OF PCBs

PCBs have produced adverse toxic reactions, such as enlargened kidneys, hydropericardium, breeding failure, thymus atropy and body weight loss in experimental animals (Hutzinger et al., 1972; Gardner et al., 1973; Goto et al., 1974; Hass et al., 1977; Safe et al., 1975; Kato et al., 1980; and McConnell, 1985). Chloracne and related dermal problems, as well as endocrine and reproductive dysfunctions have occurred in man (Mes, 1986; Kutz and Strassman, 1976; Safe, 1987; Price and Welch, 1972; Kuratsune et al., 1976; Parkinson and Safe, 1987; Nishimura et al., 1977; Polishuk et al., 1977; and Masuda et al., 1978). The persistence of these potentially toxic compounds caused concern about health effects likely to result from long term, low level exposure to these toxins. To understand the basic mechanism of PCBs, pharmacokinetic modeling was proposed (Matthews and Dedrick, 1984).

PCBs are characteristically lipophilic and thus virtually insoluble in water. The lipid solubility of PCBs allows passive absorption of these compounds from the aqueous environment of the intestinal lumen through the more lipophilic cell membranes of the intestinal wall (Albro and Fishbein, 1972). The concentration gradient favors movement of the compound from the intestine to the blood at which point it is distributed to all tissues. Initial distribution, however, is largely determined by biophysical factors such as volume of tissue, tissue/blood partition ratio, absorption of proteins, and perfusion rate (Matthews and Dedrick, 1984). As a result of these factors, the accumulation and excretion of the parent PCB compounds and metabolites are similar in all animal species.

The blood transports the xenobiotic initially to the liver and muscle. High perfusion rate and affinity for the compound account for initial deposition of the compound in the liver, whereas muscle, comprising 50% of the body weight, has high tissue volume (Matthews and Anderson, 1975). Simultaneously, a distribution of the xenobiotic to adipose tissue occurs. Adipose tissue and skin were the primary storage areas for the unmetabolized parent compound due to their lipophilic nature (Burse et al., 1974; Matthews and Anderson, 1975). A flow diagram of the interrelationship between PCB concentrations and specific tissues was presented by Lutz and Dedrick (1987). At equilbrium, the concentration of PCB was specific for a particular tissue and a change in PCB concentration in one tissue caused a corresponding change in all tissues. For example, the liver appeared to be capable of metabolizing and excreting a specific PCB congener, resulting in a decrease in the concentration of that congener in all other tissues (Lutz and Dedrick, 1987). As already stated, adipose and skin were the primary storage sites for those congeners not readily metabolized and excreted. However, these unmetabolizable congeners were not confined to these storage sites. They were circulated to all tissues by the blood, and exposure of the tissues to the compound was proportional to tissue/blood ratios and concentration of the xenobiotic in those storage tissues (Matthews and Anderson, 1975).

The percentage of parent compound in each tissue increased as the degree of chlorination increased (Matthews and Anderson, 1975; Lutz et al., 1977; and Hass et al., 1977). In fact, Matthews and associates and Hass and coworkers, demonstrated that less than 10% of the total dose was excreted as parent compound. Lutz et al. (1977) found that 95% of MCB was in the blood as a metabolite in less than one hour after injection, whereas HCB persisted in the body as the parent compound and was primarily in storage. Hydroxylated derivatives of all chlorobiphenyls studied were detected in the urine and feces of the animals studied. Hutzinger et al. (1972) noted that brook trout did not excrete any metabolites or parent compounds of MCB, DCB, TCB, or HCB, suggesting the entire compound was in storage. Metabolism played a primary role in distribution and excretion of PCBs. The amount of metabolite detected in the urine and feces appeared to be inversely proportional to the degree of chlorination. However, studies have shown that the position of chlorine atoms on the biphenyl molecule plays a major part in the metabolism of the parent compound and excretion of metabolites. DeFritas and Norstrom (1974) fed White Carneau pigeons 10% Aroclor 1254 in corn oil (w/w). Results showed that ease of metabolism was related primarily to chlorine substitution patterns rather than degree of chlorination. They reported that PCBs that contained a 2,3-, 3,4-, or 2,3,6-chlorine substitutions on one phenyl ring were metabolized very rapidly. In addition to one ring substitution, they also found that an adjacent unsubstituted carbon atom was a critical factor in rapid metabolism and excretion. In their study, all three isomers have an adjacent unsubstituted carbon atom. Those PCBs which were not readily metabolized have a combination of 2,4,5-, 2,3,4-, 2,3,4,5-chlorine substitutions on both rings which supports the direct relationship between extent of chlorination and extent of metabolism. Studies to support that concept included those by Kato et al. (1980) and Sipes et al. (1979), who studied the metabolism of the hexachloro isomers 2,3,5,2',3',5'-, 2,3,6,2',3',6' 2,4,6,2',4',6'-, and 2,4,5,2',4',5'-HCB in dogs and rats. The quantity of the various HCB isomers detected in urine and feces was in declining concentration.

The primary mechanism for PCB metabolism is initial formation of an arene oxide intermediate. Chen et al. (1976) showed a simplified metabolic scheme for 2,4,5,2',5'-pentachlorobiphenyl (5PCB). Arene oxide formation occured at the position of vicinal unsubstituted carbon atoms. Mixed function oxidase cleaved the double bond of the ring to form the arene oxide intermediate. Polar compounds were then formed through spontaneous isomerization to form hydroxylated products and epoxide hydrase to form dihydrodiol products (Chen et al., 1976). In conclusion, the rate of metabolism and excretion primarily depended on the position of chlorine substitution and the degree of chlorination.

Excretion of PCBs included active processes involving specialized mechanisms located primarily in the kidney and liver with the major routes being feces and urine (Matthews and Dedrick, 1984; Lutz and Dedrick, 1987). Excretion of the parent PCBs was minimal prior to their metabolism to the more polar compounds. However, some unmetabolized parent compounds were eliminated by association with any substance that may pass through the intestine (Matthews and Dedrick, 1984). There have been many studies on the metabolism and excretion of PCB isomers in numerous species. Table 1 lists isomer, species and reference.

The pharmacokinetics of PCB distribution can be described in a mathematical model using the liver as an example:

$d/dt(VlCl)=Ql[Cb-Cl/Rl]-Km(Cl/Rl)$, (Matthews and Dedrick, 1984) where t=time, V=tissue volume or mass, C=concentration, Q=blood flow rate, Km=metabolic clearance, R=equilbrium tissue/blood ratio, l=liver, b=blood.

For a compartment in which metabolism does not occur, such as adipose tissue, the differential equation took the form:

$d/dt(VaCa)=Qa[Cb-Ca/Ra]$, (Matthews and Dedrick, 1984)

TABLE 1 PCB ISOMERS STUDIED IN VARIOUS SPECIES

ISOMER	SPECIES	REFERENCE
4-monochlorobiphenyl, (MCB) 4,4'-dichloro, (DCB) 2,4,5,2',5'-pentachloro, (PCB) 2,4,5,2',4',5'-hexachloro, (HCB)	rat	Lutz et al., 1977
2,5,2',5'-tetrachloro, (TCB)	rabbit	Gardner et al., 1973
2,4,5,2',4',5'-HCB	rat, rabbit mouse	Sundstrom et al., 1976
4-MCB 4,4'-DCB	goat	Hass et al., 1977
4-MCB	goat	Safe et al., 1975
4,4'-DCB	cow	Safe et al., 1975
4-MCB 4,4'-DCB 2,5,2',5'-TCB 2,4,5,2',4',5'-HCB	pigeon, rat and brook trout	Hutzinger et al., 1972
4-MCB 4,4'-DCB 2,4,5,2',5'-PCB 2,4,5,2',4',5'-HCB	rat	Matthews and Anderson, 1975

where a=adipose and the other symbols are the same as above.

The prediction of distribution included substantial parameters. Categorizing parameters into four groups simplifies the above equations.

1. anatomic - tissue size and organ volume
2. thermodynamic - equilibrium partitioning or binding coefficients
3. physiologic - metabolism, blood flow rates, rate constants and clearance
4. transport - diffusion coefficients and cell membrane permeabilities

The assumption for both differential equations was that the PCB in the blood was in equilibrium with the PCB in the tissue (Matthews and Dedrick, 1984).

ENZYME INDUCTION

Cytochrome oxidase and oxygenase are two mitochondrial enzymes which utilize oxygen in the body. The larger of the two systems, cytochrome oxidase, reduces over 90% of the oxygen consumed by most cells. A more complex and specialized system occurs in the endoplasmic reticulum (Lehninger, 1982). This microsomal monooxygenase system, also referred to as the hepatic microsomal drug metabolizing enzyme, is located in the liver (Poland and Glover, 1977). These enzymes catalyze reactions in which only one oxygen atom, hence monooxygenase, is incorporated into the organic substrate molecule. The other oxygen atom is reduced to water. The enzyme complex consists of NADPH + H^+, cytochrome P_{450}, O_2, and a lipid soluble compound.

Both cytochrome oxidase and cytochrome P_{450} react with oxygen and carbon monoxide. However, cytochrome P_{450} is differentiated from cytochrome oxidase in that the reduced cytochrome P_{450} carbon monoxide complex absorbs light strongly at 450 nm. The 3-digit number usually represents wavelength absorption of light for specific cytochromes i.e., cytochrome P_{448} absorbs light at 448 nm (Lehninger, 1982).

Cytochrome P_{450} catalyzes hydroxylation reactions in which a lipid soluble compound, in this case a PCB congener, becomes a hydroxylated product. As a result of the attachment of a polar group, it can undergo excretion because it becomes more water soluble. The main substrate accepts one of the oxygen atoms to produce the hydroxylated product. Cytochrome P_{450} serves as the monooxygenase enzyme which is the catalyst in the reaction. The cosubstrate, NADPH + H^+, provides the H atoms to reduce the other oxygen atom to H_2O. Various experimental studies have been done to detect activity of cytochrome P_{450} when induced with toxins. An increase in the concentration of cytochrome P_{450} was detected in the livers of barn owls and chicks (Rinzky and Perry, 1983). Aroclor 1254 was injected once into the owl's liver parenchymal tissue at a dose of 30 mg/kg body weight and fed at 10 mg/kg body weight/day for 7 days to the chicks. There was a significant rise in the catalytic activity of cytochrome P_{450} in the barn owl and in the chick (Rinzky and Perry, 1983). In another study, Aroclor 1254 was equipotent as an inducer of O-demethylase and aniline hydroxylase activities in the livers of rats and mice (Ehrich and Larsen, 1983). In the chicken, however, aniline hydroxylase activity increased over 2-fold; whereas, only a minimal, yet statistically significant increase was observed in O-demethylase activity (Ehrich and Larsen, 1983). According to studies by Cecil et al. (1987), Aroclor 1254 appeared to be the best of 4 Aroclors as an inducer of liver microsomal enzyme activity in the male chicken when compared to Aroclor 1221, 1242 and 1268. Results showed that these Aroclors did not increase liver weight when fed at 5 ppm but produced an increase in aminopyrine demethylase activity. Also, the higher chlorinated compounds, Aroclors 1254 and 1268, produced increases in both liver weight and aminopyrine demethylase activity per mg of protein when fed at 50 and 500 ppm to chickens. Finally, chickens fed 500 ppm Aroclor 1268 showed increased aniline hydroxylase activity per mg of protein. The data indicated that the induction of liver microsomal enzymes appeared to be a more sensitive response parameter then the increase in liver weight (Cecil et al., 1987). Hamilton et al. (1983) were interested in determining the rate of basal metabolism and the inducibility of mixed function oxidase (MFO) enzymes during early stages of development on the chick embryo. Concern arose because metabolism of xenobiotics during this critical period of embryogenesis could have serious developmental consequences. They showed that the chicken embryo at 3 days of incubation had basal aryl hydrocarbon hydroxylase (AHH) activity comparable to adult levels. In addition, they noted that at the third day of incubation it was possible to assay activity, a full day prior to liver differentiation. Aryl hydrocarbon hydroxylase activity was inducible at 5 days of incubation by 3,4,3',4'-tetrachlorobiphenyl (TCB). At 7 days of incubation, a 14-fold maximum induction was reached which was equivalent to adult levels. Incubation days 17 to 21 were studied separately due to the sharp increase in induction. Within 3 hours, TCB began to induce AHH activity on day 17 of incubation. Maximal induction was reached within 6 to 9 hours and continued for at least 96 hours of incubation. Chicks that were between 1 and 3 days old showed the highest levels of activity. There was a 3-fold increase in basal MFO activity in chicken liver after hatching and then a slight decline to normal adult levels by day 7. A final observation was dose-response kinetics for AHH induction by TCB. It was similar in all ages observed. These researchers concluded that early in development, the chicken embryo has an active MFO system which is highly inducible (Hamilton et al., 1983). Rifkind et al. (1984) did similar studies with chick embryo livers determining the induction of MFO by various individual PCB congeners. The 4 highly purified congeners investigated were 3,4,3',4'-tetra (TCB), 3,4,5,3',4',5'-hexa (345-HCB), 2,4,5,2',4',5'-hexa (245-HCB), and 2,3,6,2',3',6'-hexachlorobiphenyl (236-HCB). Twenty-four hours after administration, data showed that TCB and 345-HCB were 3 to 4 fold more potent than 245-HCB both as hepatotoxins and as inducers of the cytochrome P_{448} mediated MFOs, AHH and 7-ethoxyresorufin deethylase. The fourth congener, 236-HCB, was inactive as an inducer as well as a hepatotoxin. Poland and Glover (1977) noted two structural requirements which appear to be necessary for induction of AHH activity: 1). the biphenyl congener must have adjacent chlorine atoms in at least two lateral positions of each benzene ring, i.e. 3,4,3',4'-tetrachlorobiphenyl and 2). the biphenyl congener cannot have chlorine substitutions in positions adjacent to the biphenyl bridge which are the 2,6,2'or 6'positions. Poland and Glover (1977) studied 16 biphenyl congeners to determine induction of hepatic AHH activity in the chicken embryo. Enzyme activity was measured 24 hours after administration of each congener. Only 3 of the 16 compounds induced activity: 3,4,3',4'-TCB; 3,4,5,3',4',5'-HCB; and 3,4,5,3',4',5'-hexabromobiphenyl, all being equipotent. The 3 congeners have the criteria needed for induction of AHH. They also found that when rats were injected with a single intraperitoneal dose of Aroclor 1254, (500 mg/kg body weight), AHH activity had a > 1000% increase and aminopyrine N-demethylase (AND) had a 250% increase in activity. Evidence indicated Aroclor 1254, a commercial mixture of chlorobiphenyls, caused induction of a mixed pattern of microsomal monooxygenase activities due to its composition of many isomers. They concluded that a specific congener may induce AHH activity or AND activity, or show a lack of induction but never will one congener cause induction of both enzymes. On the other hand, Aroclor 1254, can cause induction of both enzymes due to its mixture of isomers.

AVIAN TOXICITY

Aroclor 1254, at concentrations ranging from 0 to 440 ppm was fed to Bengalese finches to determine the toxicity of PCBs to this species (Prestt et al., 1970). An early sign of PCB poisoning was a lack of balance while perching. Later, three birds showed paralysis of the legs. Before death, a trembling of the body and shaking of the wings were reported. All birds that died had reduced body weights as a result of anorexia. Enlargement of the kidneys and hydropericardium were also reported. The liver, however, remained normal in size. This is due to, they believe, the liver and fat reserves not being completely used. When a bird lacks an appetite during illness or is starved experimentally, the PCB tends to transfer from the fat reserve to the brain. The concentration in the liver tends to increase due to loss of organ weight and body fat. In simpler terms, the PCBs circulate in the body when fat cells decrease in size, and the PCBs accumulate in the liver for possible detoxification and metabolism (Polin et al., 1986). PCB toxicity became a concern to many scientists when the persistence of the compound was better understood. There was a concern for the fate of wild birds knowing a bird of prey population existed. Prestt et al. (1970) reported that the highest concentration of PCBs appeared to be in the bird-feeding and fish-feeding species. They also found in a comparison study of specific predator feeding, that freshwater fish-feeders appeared to contain the highest concentration of PCBs followed by bird-feeders and then mammal-feeders. The environmental persistence of PCBs make it almost impossible to avoid exposure of the predators to the toxins; thus these toxins are a contributor to the everlasting problem of breeding failure among predatory species (Prestt et al., 1970). They do conclude however, that PCBs are unlikely to have caused widespread lethal toxicity among wild predatory birds in Britain. The population declines were most severe in the south and east of Britain which were a result of lethal poisoning by aldrin, dieldrin and heptachlor (Jeffries and Prestt, 1966).

In general, PCBs have a low acute toxicity for birds (Peakall, 1986). The 5 day LC_{50} value of Aroclor 1254 for Japanese quail, Bobwhite, Ringneck dove and Mallard duck are 4844, 1175, 1312 and 2798 ppm, respectively (Heath et al., 1972). A steady increase in toxicity as chlorination increased was observed in the birds studied. Heath et al. (1972) suggested that toxicity is not determined solely by percent chlorine. Among various subacute studies, Peakall (1986) observed the chicken to be the most sensitive to Aroclor 1254 when compared to Japanese quail, Bobwhite, pigeon, Ringneck dove, mallard, pelican and American kestral. There were no mortalities observed in the studies except for the chicken. Dietary levels of 400 ppm Aroclor 1242 resulted in 100% mortality in chickens after 28 days (Dieter, 1974).

Aroclor 1242 appeared to be the most toxic to chickens when compared to Aroclor 1254 with 40% mortality after 38 days (Dieter, 1974). Dieter (1974) also noted that chickens fed Aroclor 1254 at concentrations of 200 and 400 ppm for 28 days resulted in 20% and 60% mortality, respectively.

The effect of PCBs on avian reproduction were variable among species. Peakall (1986) presented a complete list of different species affected by different Aroclors. Many studies were done to confirm that PCBs had no effect on egg shell thickness (Custer and Heinz, 1980; Heath et al., 1972; Platonow et al., 1973; McLane and Hughes, 1980; Peakall, 1971; Riesbrough and Anderson, 1975; Cecil et al., 1973; Dahlgren and Linder, 1971; and Scott, 1977). Young male chickens fed Aroclor 1254 at a level of 250 ppm showed a marked reduction in comb size within 6 weeks of exposure and the weights of the testes were reduced after 9 weeks (Platonow et al., 1973). Ahmed et al. (1977) observed a decreased semen volume, sperm concentration and testes weight in White Leghorn male chickens from feeding 10 to 40 ppm of Aroclor 1254. American kestrals were fed 33 ppm Aroclor 1254 which caused a significant decrease in sperm concentration without affecting semen volume (Lincer and Peakall, 1970). Embryonic mortality and chromosomal alterations were studied in Ringneck doves fed 10 ppm Aroclor 1254 (Peakall et al., 1972). The first generation of eggs from the contaminated parents of the Ringneck doves was normal. However, the second generation had low hatching success due to embryonic mortality. Relative frequencies of chromosomal aberrations suggested that PCBs had a clastogenic action in dove embryos. Additional studies were done by Bitman et al. (1972) who reported the effects of Aroclor 1242 on liver vitamin A in rats and quail. Both species were fed 100 ppm Aroclor 1242. The animals had an increase in liver weight and liver lipids and a striking decrease (approximately 50%) in the liver concentration and content of vitamin A. Other studies of PCBs indicated that feed intake, egg production and hatchability of chickens and quail were decreased when fed high dietary concentrations of polybromobiphenyls (PBBs) which are structurally similar to PCBs (Ringer and Polin, 1977).

HUMAN TOXICITY

In general, PCBs in human populations are probably the result of life long or chronic exposure (Mes, 1986). Exposure to this persistent contaminant begins as early as fetal development *in utero* as shown by the presence of PCB residues in embryonic and fetal tissues (Nishimura et al., 1977; Polishuk et al., 1977).

Post-natal exposure to PCBs will continue in those infants breast-fed with contaminated mother's milk (Wickizer et al., 1981). The highest levels of PCBs in human milk were found in Spain, Germany and Michigan. The average level of PCB in breast milk from all countries tested is 37 ppb on a whole milk basis (Mes, 1986).

Several studies have reported relatively high levels of PCBs in serum and adipose tissues of humans occupationally exposed to commercial mixtures of PCBs. Common occupations involving exposure include capacitor and transformer maintenance and repair, railway car maintenance, refuse workers, paint manufacturers and an occupation in any company having machinery or equipment containing PCBs (Safe, 1987; Kutz and Strassman, 1976; Mes, 1986; Price and Welch, 1972).

Since experimental studies involving human exposure to PCBs have not been done, PCB toxicity to humans cannot be established with certainity. However, information is available from accidental exposure of humans in Japan to PCBs resulting from an incident involving rice oil contamination (Kuratsume et al., 1976). Price and Welch (1972) reported that 41 to 45% of the general population has adipose tissue levels of 1.0 ppm or more of PCBs with isomers from Aroclor 1254, 1260, 1262 and 1268. The chlorobiphenyls ranging from pentachloro- to decachlorobiphenyls have been identified in adipose tissue. Kutz and Strassman (1976) also found levels of 1.0 ppm or more of PCBs in human adipose tissue and in milk samples collected from 35 to 40% of the general population, respectively.

In 1968, an epidemic of a peculiar skin disease was reported in Japan. The victims had ingested a brand of rice that had been accidentally contaminated with Kanechlor 400, a commercial brand of PCBs. The disease was called "Yusho", namely "oil" disease. A similar accident occurred in 1979 and these victims were referred to as "Yu-Cheng" (Kuratsume et al., 1976). The most common initial symptom was chloracne and related dermal problems. In addition to headaches, stomach aches, numbness of extremities, coughing, bronchial disorders and joint pains were common subacute symptoms (Mes, 1986). Follow-up studies revealed a gradual recovery from the skin problems (Urabe and Asahi, 1984). The severe acute and chronic effects of PCBs observed in these victims were not observed in the occupationally exposed populations and the general population (Mes, 1986).

Parkinson and Safe (1987) having reviewed several studies, compiled a list of clinical signs indicative of PCB toxicity in different species:

1). A wasting syndrome which is characterized by a progressive weight loss not fully explained by decreased food consumption.

2). Skin disorders which include chloracne (acne form eruptions), alopecia, edema, hyperkeratosis and blepharitis.

3). Hyperplasia of the epithelial lining of the extrahepatic bile duct, gall bladder and urinary tract.

4). Lymphoid involution due to thymic and splenic atrophy.

5). Hepatomegaly and liver damage which includes necrosis, hemorrhage and intrahepatic bile duct hyperplasia.

6). Porphyria due to disorders in porphyrin metabolism of the cutanea tarda type.

7). Endocrine and reproductive dysfunction including altered plasma levels of steroid and thyroid hormones, menstrual irregularities, reduced conception rate, early abortion, excessive menstrual and postconceptional hemorrhage and anovulation in females. Males showed a testicular atrophy and decreased spermatogenesis.

8). Teratogenesis including cleft palate and kidney malformations.

9). Carcinogenesis including hepatocarcinoma.

PROCESSING OF TISSUES

One to 10 gram samples of finely ground whole budgie, feathers included, or tissue were blended in a Waring commercial blender with 12.5 ml of toluene:ethyl acetate (1:3)/g sample. The sample was blended at low speed for one minute. The fluid portion was decanted through a medium size ceramic Buchner funnel with two sheets of Whatman filter paper saturated with solvent. A 500 ml filter flask was connected to a vacuum for collecting the fluid. The blending and filtering steps were repeated two additional times. In the final step, all of the extract was poured into the funnel and the blender was rinsed thoroughly with solvent. A glass powder funnel was plugged with glass wool and filled three-quarters with anhydrous, granular sodium sulfate (Mallinckrodt). The supernatant was poured through the Na_2SO_4 to dehydrate it. The solution was then poured into a graduated cylinder to measure its volume. If the volume of the solution exceeded 250 ml, then only 10% of the sample was used for rotavaporization, otherwise all of the sample was rotavaporized. The Buchi rotavapor system evaporated the solvent leaving behind fat, higher molecular weight compounds, and the xenobiotic. Distillation of solvents began when the water bath reached a temperature in the range of 35 to 50 °C. Evaporization continued until 5 ml of solution remained in the flask. The solution was then transferred to a 10 ml volumetric flask. All glassware was rinsed with solvent and the rinse transferred to the 10 ml flask. The volume was adjusted to 10 ml with solvent. The 10 ml sample was then processed through an ABC Labs Autoprep 1001 gel permeation chromatograph (GPC). Bio Beads S-X3, 200-400 mesh were packed in the column and toluene:ethyl acetate (1:3) was the solvent used in the system. The GPC cleaned a maximum of 24 samples at one time. A 5 ml plastic column was placed into each flask containing a sample, and the main column was placed into the solvent reservoir. One sample was washed at a time with the solvent. The higher molecular weight compounds tend to be washed out during the cycle. The preset parameters for the specific solvent were: dump the sample for 21 minutes; collect the sample for 15 minutes; and wash the sample for 5 minutes at a flow rate of 5 ml/min. The collected sample was evaporated a second time to 5 ml and transferred to a 10 ml volumetric flask. All glassware was rinsed with solvent again, and the fluid was transferred to the flask until the volume reached 10 ml. The solution was then transferred to a 15 ml screwcap test tube for subsequent analysis on an electron capture gas chromatograph (GC).

GC analysis was conducted using a Varian Model 3700. A glass voormen silyated column was packed with 3% OV-1, 100-200 mesh, chromosorb W, H.P. An electron capture detector was used. GC parameters were: column temperature=200 ^{o}C; injector temperature=220 ^{o}C; detector temperature=270 ^{o}C. Carrier gas was 99.9% ultra pure nitrogen with a flow rate of 40 ml/min. PCB quantitation was done by measuring the height of 6 major peaks having retention times ranging from 4 to 10 minutes. Standard solutions were prepared from Aroclor 1254 (Monsanto Chemical Company, St. Louis). The height of the 6 major Aroclor 1254 peaks were measured in millimeters for all tissues. The number assigned to each peak is the distance in millimeters between the solvent and the individual peak on the GC. Calculations for standards are shown in Appendix I. A standard curve was constructed daily using the sum of the 6 major Aroclor 1254 peaks. A correlation of 85% or better was accepted for sample extracts from those standards (Batty et al., 1990).

EXPERIMENTAL OUTLINE-I

Fifty American budgerigars (budgies) approximately 16 months of age were used in the study. A 5 X 5 Latin square design was used containing 5 treatments and 10 budgies in each treatment. Two batteries were used, each consisting of 25 metal bird cages, 21H X 21W X 27L mm. There were 5 rows and 5 columns and each row, column, and diagonal contained one bird per treatment with no replicates. Treatments consisted of 0.0, 12.5, 25.0, 50.0, and 100.0 ppm Aroclor 1254. Budgies were provided water and feed *ad libitum* and were fed the treatments for 28 days. Feed intake was recorded weekly, and any spilled feed on the bottom of the cage was collected and weighed. Body weights were recorded on day 1 and day 28 of the experiment. Daily observations were made to detect any adverse effects on behavior or health. Mortality was recorded and dead birds were necropsied to detect any abnormalities. Feed was removed 18 hours prior to termination of the trial to insure an empty digestive tract. Birds were euthanized by decapitation and blood, liver, kidney, skin, muscle and the brain were collected from 5 birds in each group. The other 5 birds were pooled for whole carcass residue analysis. Samples of the diet from each treatment level were collected for determination of actual Aroclor 1254 concentration. Table 2 represents the dietary treatments, the dietary code system and the number of birds in each treatment. The first and second

TABLE 2 TREATMENT GROUPS AND LABELING SYSTEM FOR EXPERIMENT I OF BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254	LABEL	# BIRDS
0.0 ppm	87-09-01	10
12.5 ppm	87-09-02	10
25.0 ppm	87-09-03	10
50.0 ppm	87-09-04	10
100.0 ppm	87-09-05	10

number in the dietary code represented the year and the month, respectively, at the time the experiment was started. The third number represented the specific treatment group.

PREPARATION OF THE DIET-I

Aroclor 1254 was purchased from Monsanto Chemical Company. Exactly 428 mg of Aroclor 1254 were weighed into a tared beaker. It was then dissolved in 15-20 ml hexane. Approximately 25 kg of Dr. D's Budgie Maintenance crumbles (Avi-Sci, Inc., Okemos, MI) was ground to a finer texture. Exactly 100 g of the finely ground budgie diet was sifted through a #20 U.S.A. Standard Testing Sieve onto laboratory craft paper. Working under a laboratory fumehood, the PCB/hexane solution was pipetted, drop by drop, onto the 100 g of diet. The beaker was rinsed thoroughly 3 times with hexane and the hexane rinses were pipetted onto the diet. After allowing most of the hexane to evaporate, the sample of feed was evenly mixed with a metal spatula by rolling the diet over the instrument several times. The 100 g of diet was sifted through the sieve for three times. Exactly 4.15 kg of budgie diet were weighed into a container and the 100 g PCB diet was added to it. The container was then covered with a prelabeled lid. This 100 ppm stock diet was tumbled for 10 to 15 minutes in a modified paint mixer to assure mixing. A 2.2 kg portion of the 100 ppm stock diet was transferred into a labeled can. This was treatment #5 designated as 87-09-05. Each treatment was prepared from a dilution of the 100 ppm stock diet with uncontaminated budgie diet. Treatment #4 consisted of 50 ppm made from 1.1 kg of PCB stock diet and

1.1 kg of clean budgie diet and was then mixed and tumbled for 15 minutes. Treatment #3, (25 ppm), was made from 0.55 kg of PCB stock diet and 1.65 kg clean budgie diet and was then mixed and tumbled for 15 minutes. The second treatment, (12.5 ppm), was made from 0.275 kg of PCB stock diet and 1.925 kg of clean diet and was mixed and tumbled for 15 minutes. The control treatment (#1) was 2.2 kg budgie maintenance diet and designated 87-09-01.

EXPERIMENTAL OUTLINE-II

Fifty American budgerigars (budgies) approximately 16-24 months of age were used in the second experiment. Table 3 represents the dietary treatments, the dietary codes, and the number of birds in each treatment. This 28-day experiment was conducted to investigate the effects of dietary Aroclor 1254 at levels of 0.0 to 100.0 ppm on liver enzyme induction. The specific enzyme assayed was 7-ethoxyresorufin-O-deethylase. Flight cages, 20H X 20W X 32L, were used to house 10 birds per treatment in each cage. Water and feed were offered *ad libitum*. A light cycle of 15 hours light:9 hours dark was used. Temperature was maintained at 65-70 ^{o}F by an electric heater. Daily observations of birds were made to monitor any adverse effects from feeding the contaminated diet. Feed intake and body weights were recorded weekly. Mortality was recorded and dead birds were necropsied to examine internal organs and tissues for abnormalities. Budgies were euthanized by cervical dislocation and livers were immediately removed and weighed in preparation for microsomal isolation.

TABLE 3 TREATMENT GROUPS AND LABELING SYSTEM FOR EXPERIMENT II OF BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254	LABEL	# BIRDS
0.0 ppm	89-03-01	10
12.5 ppm	89-03-02	10
25.0 ppm	89-03-03	10
50.0 ppm	89-03-04	10
100.0 ppm	89-03-05	10

PREPARATION OF THE DIET-II

Aroclor 1254 was purchased from Monsanto Chemical Company, St. Louis, Missouri. Exactly 829 mg Aroclor 1254, were weighed into a tared beaker. It was dissolved in 15-20 ml hexane. Twenty-five kg Dr. D's Budgie Maintenance diet (Avi-Sci, Inc., Okemos, MI) was weighed and approximately 100 g of the 8.25 kg was sifted through a #20 U.S.A. Standard Testing Sieve onto craft paper. Working under a laboratory fumehood, the PCB/hexane solution was pipetted, drop by drop, onto the 100 g of diet. A metal spatula was used to roll the PCB solution into the diet. After allowing most of the hexane to evaporate, the PCB diet was sifted through the sieve a total of three times. The 8.15 kg budgie diet that was previously weighed was kept in a plastic barrel. The 100 g of PCB stock diet was divided into two metal cans to be tumbled on a modified paint mixer for 20 minutes to assure mixing. A smaller can and lid was labeled "89-03-05, 100 ppm PCB". After 20 minutes, 4.4 kg of the 8.25 kg stock diet was transferred into the "100 ppm PCB" can. Into a second can labeled "89-03-04, 50 ppm PCB", a 2.2 kg portion of the 100 ppm stock diet was weighed and mixed with 2.2 kg of clean budgie diet. It was then tumbled for 20 minutes. A 1.1 kg portion of the stock diet was mixed with 3.3 kg of clean budgie diet and tumbled for 20 minutes in a 3rd can labeled "89-03-03, 25 ppm PCB". A 0.55 kg portion of the stock diet was mixed with 3.85 kg clean budgie diet and tumbled for 20 minutes in a 4th can labeled "89-03-02, 12.5 ppm PCB". A "control diet" consisted of clean budgie diet. A 4.4 kg portion was weighed and transferred into a can designated as "89-03-01, 0.0 ppm PCB".

MICROSOMAL ISOLATION

Livers from 2 to 3 budgies were pooled to obtain at least 1 gram of liver, weighed accurately. The weighed livers, kept cold over ice, were placed into a polycarbonate centrifuge tube and minced into small pieces with scissors. Approximately 10 ml of cold homogenizing buffer, Trizma base KCl, and double-distilled water (DDH_2O), were then added to the tube. The cellular membranes were ruptured by the shearing forces of a Polytron Homogenizer (Type PT 10 OD). The livers were homogenized twice for 5 seconds on speed 5. In between homogenizations, the blade was rinsed with DDH_2O following removal of connective tissue and blood vessel fragments. Samples were centrifuged for 20 minutes at 12,000 rpm in a Sorvall Superspeed RC-2 centrifuge with a SA-600 rotor. The resultant supernatant, fraction 1, was poured through a triple layer of cheese cloth into a thick-walled polycarbonate tube. Samples were centrifuged a second time for 75 minutes at 25,000 rpm in a Beckman Ultracentrifuge with a SW-28 rotor. The soluble fraction of cytoplasm was discarded and 10 ml of 200 mM tris-HCl was added to the microsomal pellet in the tube. Samples were covered with parafilm and stored in a freezer at -25 ^{o}C (Lehninger, 1982).

LOWRY'S PROTEIN ASSAY

The quantity of cellular protein present in the resuspended microsomal cells was determined by the method from Lowry et al. (1951). The methods pertaining to preparation of all reagents are listed in Appendix K.

This assay was to standardize protein concentrations so that 1 ml of the microsomal preparation contained 1 mg of protein. The microsomal sample tubes were thawed, vortexed and the initial volume measured. Duplicate borosilicate tubes were labeled for each sample in addition to labeling duplicate blank and standard tubes. Working reagents were prepared on the same day the assay was run. The following amounts of deionized, distilled water were added to blank tubes, standard tubes, and sample tubes: 1.02, 0.92,. and 1.0 ml, respectively. Bovine serum albumin (BSA) was used to make the standard of 500 ug BSA/ml deionized, distilled water. A 0.10 ml of this solution was added to the standard tubes. A 1% copper sulfate solution reagent was prepared and 1.0 ml was added to all the tubes. The tubes were vortexed and allowed to stand for 10 minutes. A 10% phenol solution was prepared and 3.0 ml were added to all tubes which were vortexed immediately and allowed to stand for 60 minutes. Samples were read at 540 nm on a dual-beam spectrophotometer, calibrated with a test tube filled with deionized, distilled water. Unknown samples were placed in the cuvette individually and values recorded. Calculations for the final protein concentrations are shown in Appendix K.

7-ETHOXYRESORUFIN-O-DEETHYLASE (EROD) ASSAY

The method used to determine microsomal cytochrome P_{450} dependent EROD activity was that of Pohl and Fouts (1980). The methods for preparation of all reagents and calculations of EROD activity are listed in Appendix L.

Following determination of protein concentration, 1.0 ml of the microsomal preparation was vortexed with a precalculated volume of Tris buffer which is made by combining Trizma base and Tris-HCl and stored on ice during the assay. Each sample had two tubes with different protein concentrations. All samples were run with duplicate tubes. The reaction began with a generating system which produced an excess amount of NADPH during the preincubation phase. This excess NADPH was needed to provide the reducing power for the reaction to occur. One ml of the generating system, 0.2 ml of the above premixture, and 0.1 ml of glucose-6-phosphate dehydrogenase, were added to all of the tubes. All tubes were vortexed and at ten second intervals, samples were placed into a Gyrotory water bath shaker at an incubation temperature of 37 ^{o}C for 10 minutes. After 10 minutes, 0.1 ml of 100 um ethoxyresorufin was added to each tube at 10 second intervals. Incubation continued for 20 minutes. The reaction was terminated by the addition of 2.35 ml chilled methanol to each tube at 10 second intervals. Tubes were incubated for an additional 5 minutes to assure termination of the reaction.

The tubes were then removed from the water bath in the original order at 10 second intervals. The samples were placed in an Omnifuge RT Centrifuge with a swing bucket rotor and spun for 10 minutes at 5500 rpm. The resultant supernatant was decanted into prelabeled glass scintillation vials and the pellet was discarded. The final step was the determination of the fluorescent compound, resorufin, in the sample. A Perkin-Elmer LS-5B Luminescence Spectrofluorometer with a dual monochronometer was used. Wavelength settings for the detection of resorufin were 550 nm for excitation and 585 nm for emission. A standard curve was established using resorufin standards. The calculations for the standard response lines are listed in Appendix L. Samples were read immediately after the standard curve was obtained using the same fluorometric settings. The volume of sample added to the cuvette was 2.5 ml. Calculations for EROD activity are shown in Appendix L.

STATISTICAL ANALYSIS

Body weight, relative organ weight, and feed consumption data were analyzed using Analysis Of Variance statistics. The level of significance chosen was *alpha*=0.05.

Microsomal protein and ethoxyresorufin were also analyzed using Analysis Of Variance and group comparison tests: Fisher PLSD, Scheffe F-test, and Dunnett T-test (Stat View 512+, Brainpower, Inc., Calabasa, CA).

RESULTS

Budgies in the control group had an average body weight of 32.9 g at the onset of Experiment I and 32.1 g at the end of the study (Table 4). Budgies that were fed the highest treatment, 100 ppm, weighed 32.4 g at the beginning and 34.6 g at the end (Table 4). The net gain or loss of body weights from day 0 to day 28 was not significant ($p>0.05$). Feed consumption for budgies ranged from an average high of 8.3 g/b/d in the control group to an average low of 6.8 g/b/d in the 25 ppm Aroclor 1254 group (Table 5). The decrease in feed consumption for all treatments as the study progressed was marginally significant ($p>0.05$), as shown in Figure 2. The mean relative liver weight of control birds was 1.79% body weight and budgies fed diets containing 50 and 100 ppm Aroclor 1254 had livers weighing 2.94% body weight and 3.04% body weight, respectively (Table 6). Thus, treatment with Aroclor 1254 significantly ($p<0.05$) increased relative liver weights in the budgies. Those birds fed the 12.5 ppm diet had livers averaging 2.0% body weight which was significantly less ($p\leq0.05$) than relative liver weights in those budgies fed the 50 and 100 ppm diets (Table 6). The dose response relationship for relative liver weights is shown in Figure 3. Relative brain weights averaged 3.3% body weight to 3.7% body weight for the treatment groups (Table 6). The small changes in relative brain weights among treatments were not

TABLE 4 BUDGIE WEIGHTS FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS IN EXPERIMENT I

DIETARY AROCLOR 1254 (ppm)	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	NET CHANGE* (g)
0.0	32.9±1.6	32.1±2.2	-0.76±1.4
12.5	32.5±2.0	31.9±3.5	-0.63±2.7
25.0	31.8±2.2	31.2±3.1	-0.54±3.1
50.0	32.4±2.5	32.3±3.4	-1.47±3.5
100.0	32.4±1.8	34.6±1.4	+1.13±2.5

*initial-final=net change
**data presented as mean±standard deviation;
sample size=10/group

TABLE 5 FEED CONSUMPTION OF BUDGIES FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS IN EXPERIMENT I*

DIETARY AROCLOR 1254

0.0 ppm	12.5 ppm	25.0 ppm	50.0 ppm	100.0 ppm
8.3±1.8**	7.6±2.2	6.8±0.8	7.5±2.4	7.5±1.4

*grams of feed consumed/bird/day
**values are expressed as mean±standard deviation; sample size=10/group

TABLE 6 RELATIVE ORGAN WEIGHTS OF BUDGIES FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS**

DIETARY AROCLOR 1254	LIVER	KIDNEY	BRAIN
0.0 ppm	$1.79 \pm 0.32_{a}$*	$0.84 \pm 0.06_{ab}$	$3.34 \pm 0.64_{a}$
12.5 ppm	$2.00 \pm 0.33_{a}$	$0.90 \pm 0.14_{ab}$	$3.36 \pm 0.59_{a}$
25.0 ppm	$2.29 \pm 0.28_{ab}$	$0.80 \pm 0.14_{ab}$	$3.67 \pm 0.24_{a}$
50.0 ppm	$2.94 \pm 0.71_{b}$	$0.76 \pm 0.11_{a}$	$3.37 \pm 0.36_{a}$
100.0 ppm	$3.04 \pm 1.10_{b}$	$0.97 \pm 0.11_{b}$	$3.45 \pm 0.18_{a}$

*values are expressed as mean±standard deviation
-sample size was 5/group
-values with the same subscript are not significantly different at $p \leq 0.05$
**organ weights are expressed as a % of body weight
[organ wt.(g)/body wt.(g)] x 100

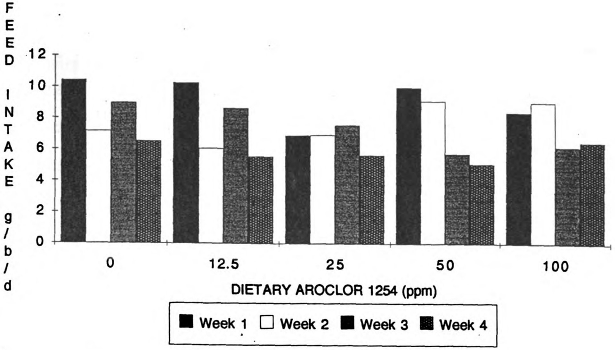

FIGURE 2 WEEKLY FEED CONSUMPTION OF BUDGIES FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

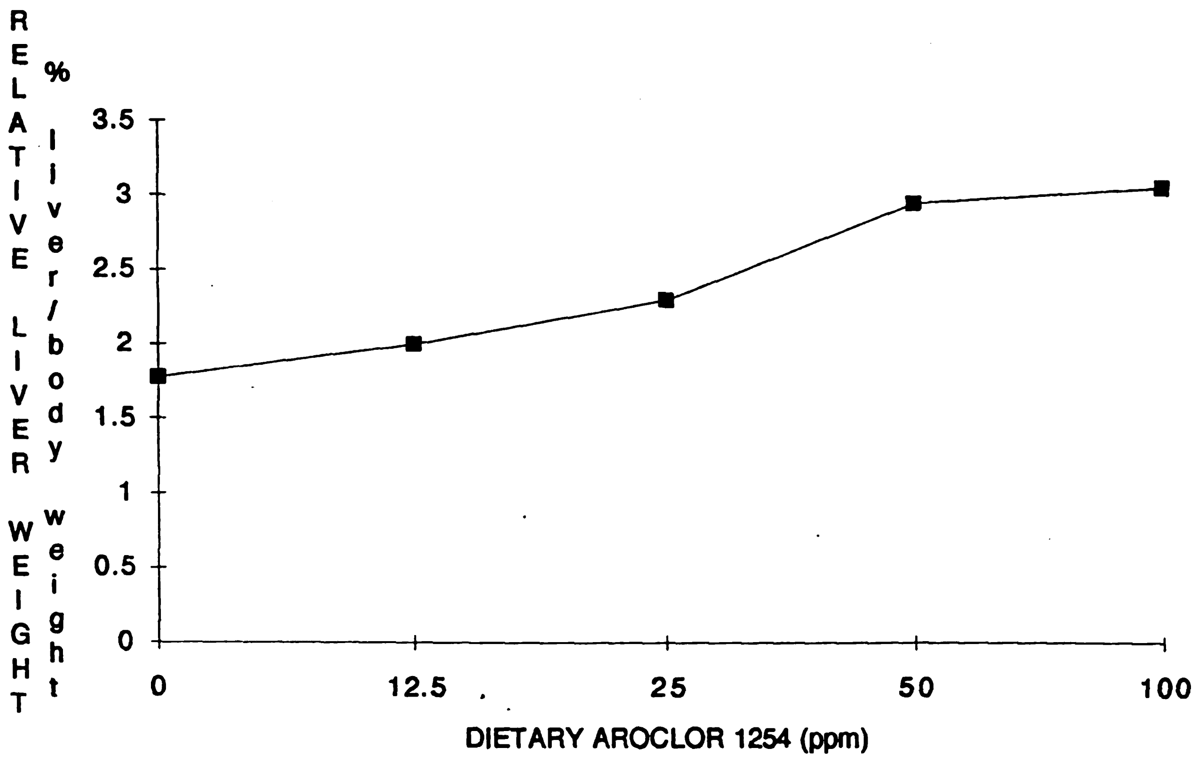

FIGURE 3 RELATIVE LIVER WEIGHTS OF BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

significant ($p>0.05$). There was no dose response relationship (Figure 4). Relative kidney weights averaged 0.84% body weight in the control group and 0.90% and 0.80% body weight in the 12.5 and 25 ppm groups, respectively (Table 6), which were not significantly different from the control value. However, those birds fed the 50 and 100 ppm diets had relative kidney weights averaging 0.76% and 0.97% body weight, respectively (Table 6). The average relative kidney weight of the 100 ppm group was significantly greater than the average relative kidney weight of the 50 ppm group. However, no treatment groups were significantly different from the control group ($p>0.05$). A dose response relationship was not apparent among the treatment groups (Figure 5).

Only 1.87 ppm Aroclor 1254 residues were measured in the carcasses of the control group (Table 7). However, the treated groups had residue levels less than the dietary concentrations of Aroclor 1254. For example, Table 7 shows that the 12.5 ppm group had residue levels of only 4.06 ppm and the 100 ppm group had levels of 44.2 ppm. All treatment groups were significantly different from the control ($p<0.05$) and a dose response relationship among groups was detected (Figure 6). There was also a dose response relationship between dietary Aroclor concentrations and the concentration of Aroclor 1254 residues in the liver (Figure 7). Residue concentration (ppm) in the liver were higher than the dietary concentrations fed to the budgies (Table 7). The lowest

TABLE 7 RESIDUAL AROCLOR 1254 CONCENTRATIONS IN WHOLE ORGANS FROM BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS*

DIETARY AROCLOR 1254 (ppm)	ORGAN	SAMPLE (ppm)**	AVE. WT. (grams)	AROCLOR 1254 in organ (ug)
	CARCASS			
0.0		1.9±0.51	32.9±1.6	61.5
12.5		4.1±0.12	32.5±2.0	132.0
25.0		10.8±0.58	31.8±2.2	344.7
50.0		10.6±0.97	32.4±2.5	342.1
100.0		44.2±16.8	32.4±1.8	1432.4
	LIVER			
0.0		0.0± 0.0	0.58±0.1	0.0
12.0		78.3±17.4	0.64±0.1	50.1
25.0		74.9± 8.9	0.70±0.1	52.4
50.0		128±43.3	0.98±0.3	125.3
100.0		296±50.8	0.88±0.2	260.2
	KIDNEY			
0.0		7.6± 0.0	0.27±0.03	2.1
12.0		14.5± 0.0	0.29±0.05	4.2
25.0		35.1± 0.0	0.24±0.04	8.4
50.0		51.2± 0.0	0.25±0.02	12.8
100.0		136± 0.0	0.33±0.03	44.9
	BRAIN			
0.0		1.0±0.03	1.1±0.2	1.1
12.0		2.0±1.61	1.1±0.1	2.3
25.0		14.5±5.21	1.1±0.1	15.9
50.0		36.1±2.18	1.1±0.1	39.7
100.0		43.9±9.13	1.2±0.1	52.7

*values represent mean±std.dev.
˜kidney samples did not contain duplicate values due to limited sample size
**carcass contained 10 birds/treatment; liver, kidney and brain samples contained 5 birds/treatment

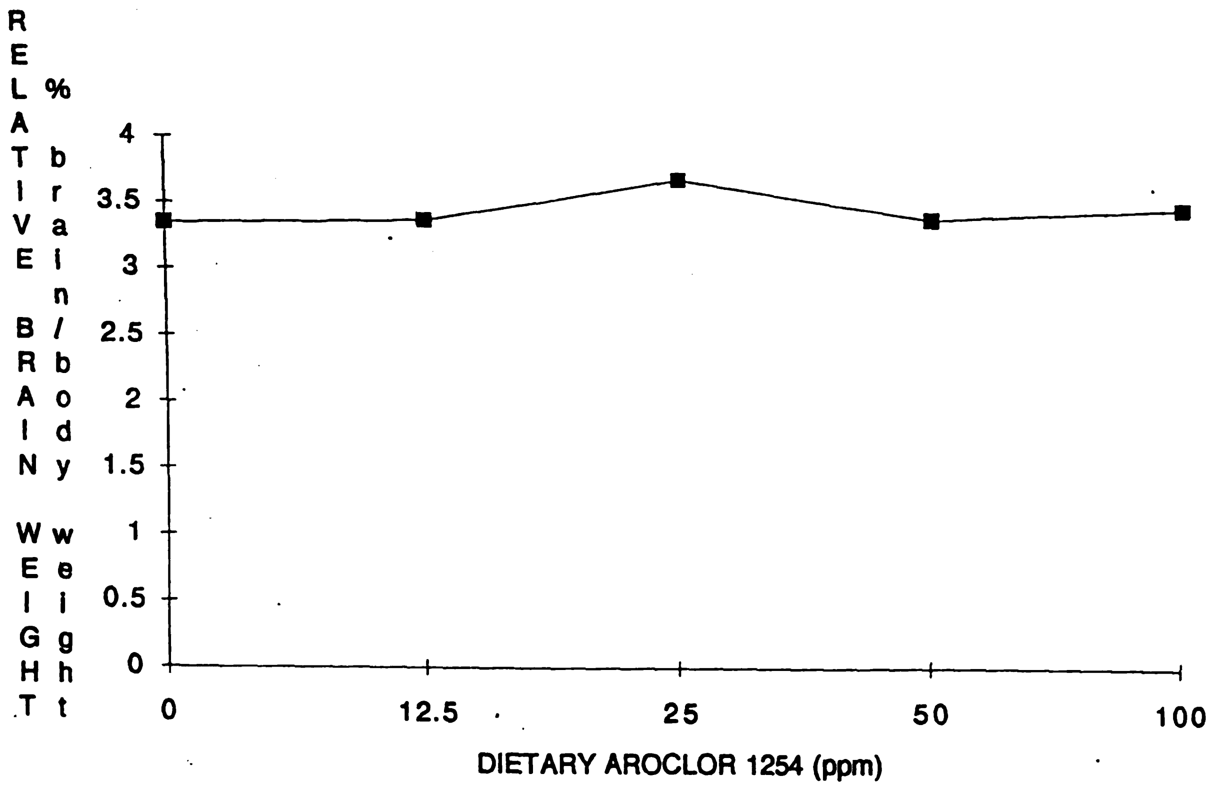

FIGURE 4 RELATIVE BRAIN WEIGHTS OF BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

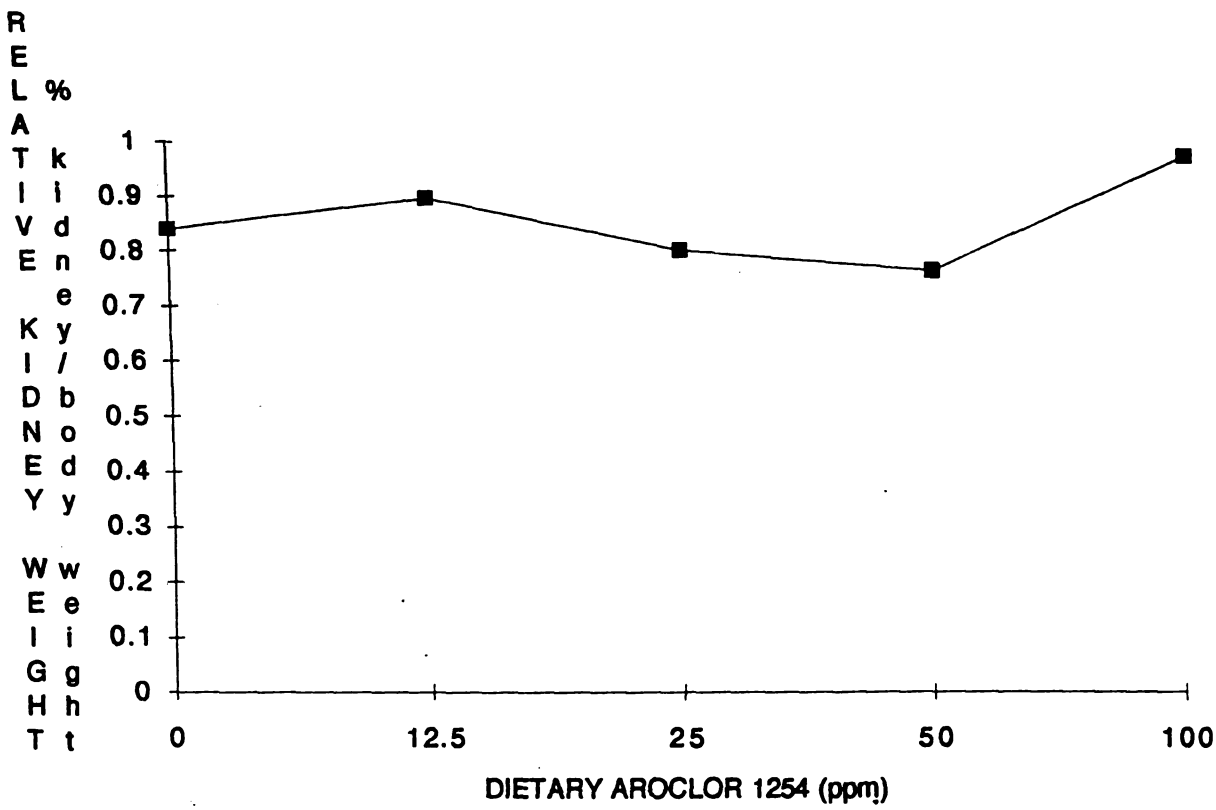

FIGURE 5 RELATIVE KIDNEY WEIGHTS OF BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

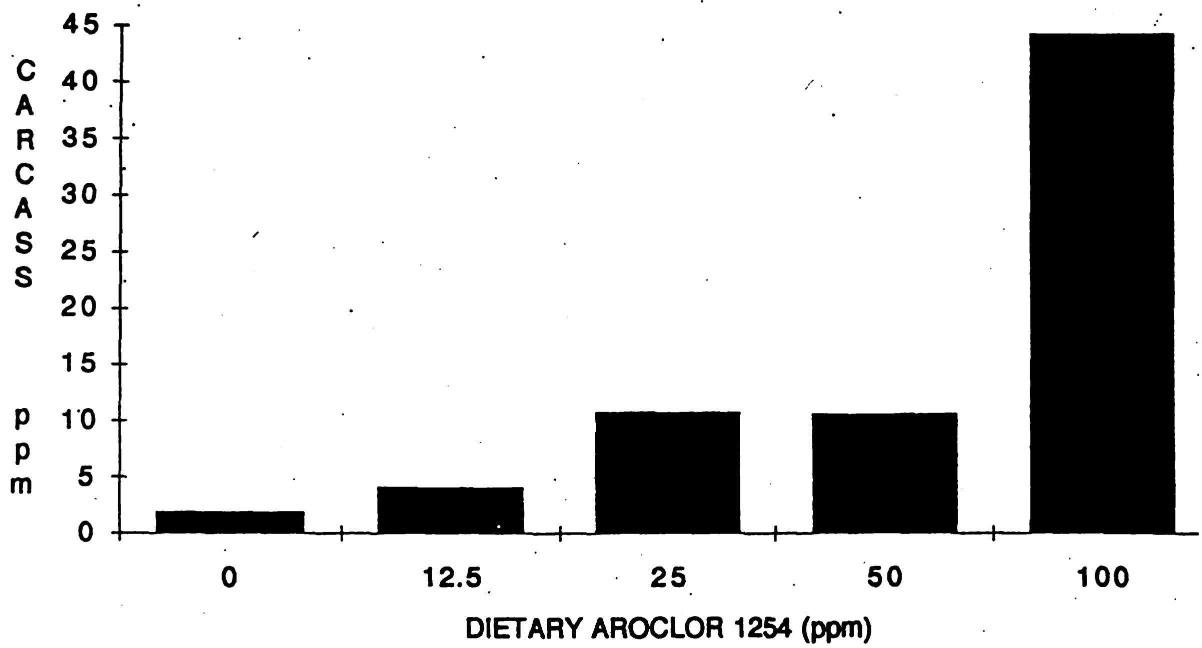

FIGURE 6 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE CARCASS FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

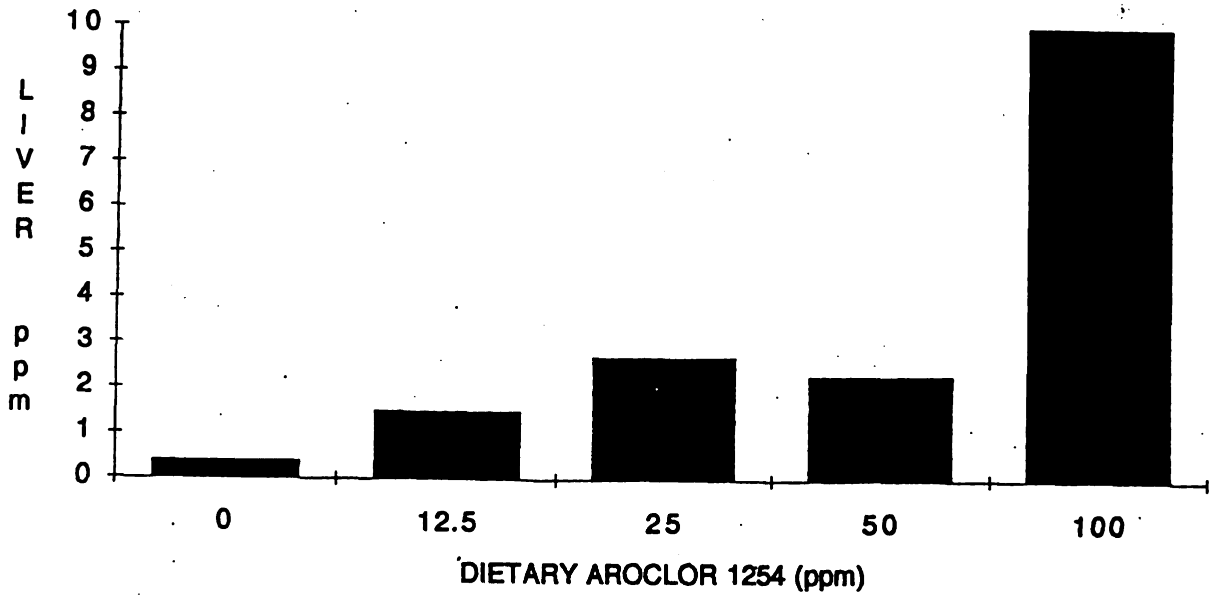

FIGURE 7 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE LIVER FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

treatment group, 12.5 ppm, had a residue concentration of 78.3 ppm and the highest group, 100 ppm, had a residue concentration of 295.7 ppm (Table 7). All treatment groups in the liver had significantly higher residue concentrations than the control group ($p<0.05$). Residue concentrations in the kidney were similar to the dietary concentrations of Aroclor 1254 with an exception of the control group (Table 7). The control group had a residue level of 7.6 ppm but the treatment groups had levels similar to dietary concentrations i.e. the 12.5 ppm group had an average residue level of 14.5 ppm and the 100 ppm group had an average residue of 135.9 ppm (Table 7). Only single pooled samples of kidney were analyzed therefore a statistical analysis was not possible. However, a dose response relationship was indicated (Figure 8). The brain had lower residue concentrations than the actual dietary concentrations. The control group had less than 1.0 ppm and the 100 ppm group had less than 44 ppm Aroclor 1254 residues (Table 7). All treatments were significantly different ($p<0.05$) and a dose response relationship is shown in Figure 9. An additional parameter for carcass, liver, kidney, and brain was measured. Since the total organ weight was known, the level of Aroclor 1254 in ug/g organ weight was calculated (Table 7). For example, the carcasses had a range of dietary Aroclor 1254 from 61.5 ug in the control group to 1432.4 ug in the 100 ppm group. Muscle, skin, and plasma were collected as partial organs, therefore only residue concentrations were

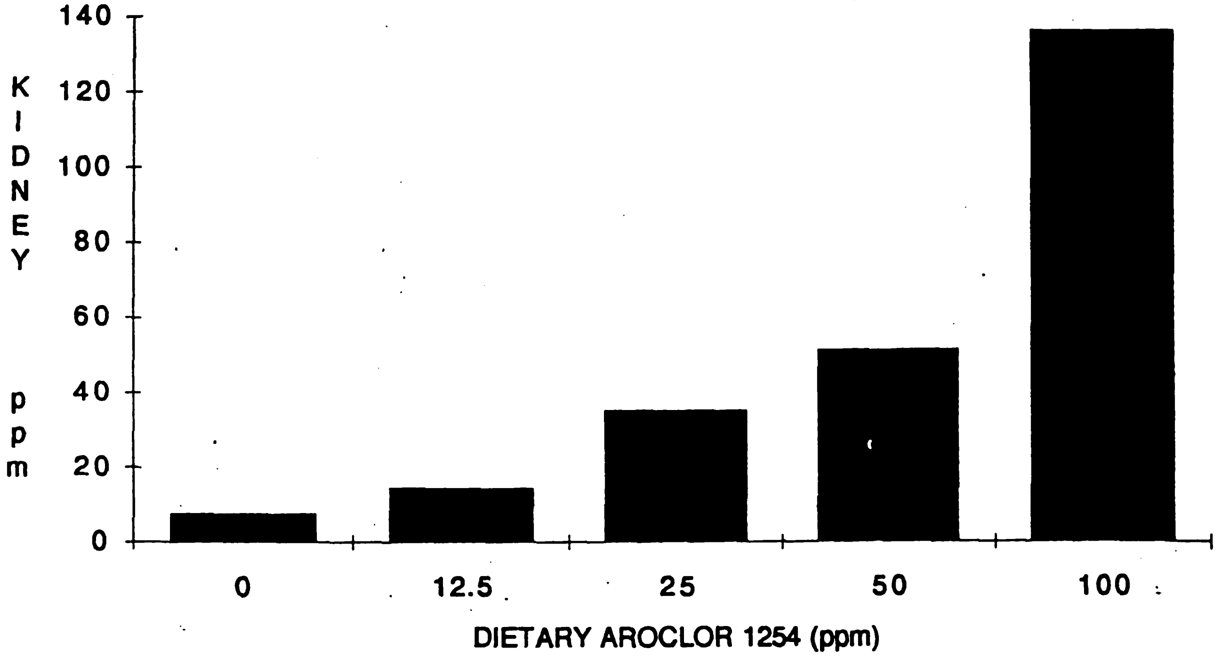

FIGURE 8 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE KIDNEY FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

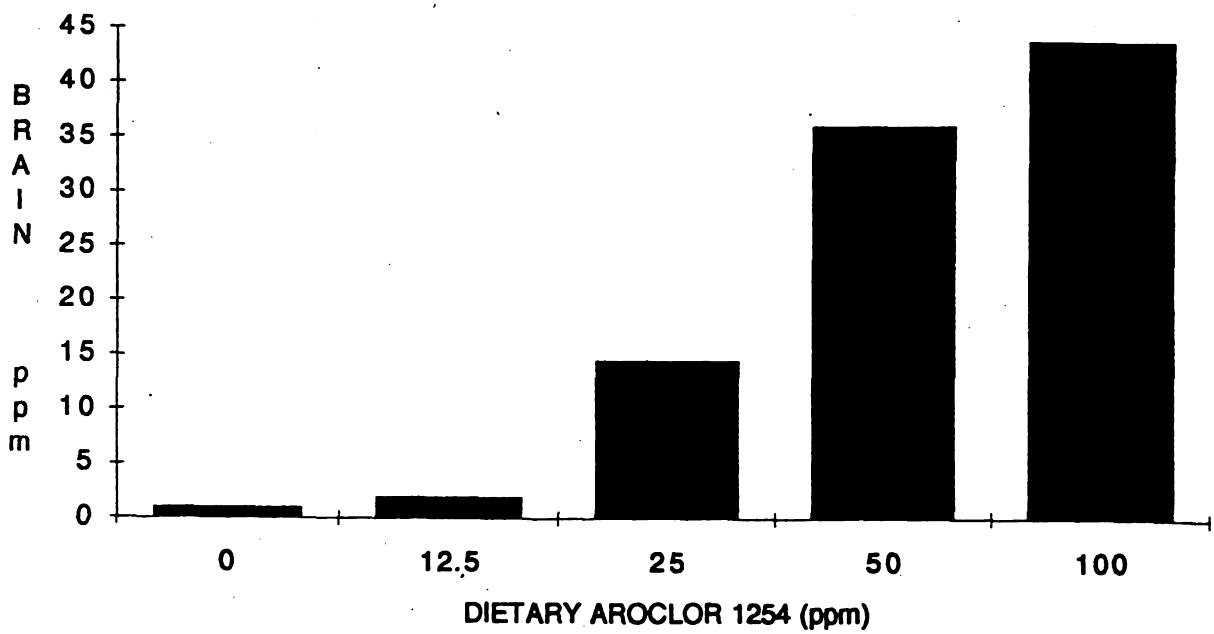

FIGURE 9 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE BRAIN FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

measured. Muscle and plasma had lower residue levels than the dietary concentrations (Table 8). The highest Aroclor 1254 residue in the muscle was only 12.5 ppm and in the plasma, 10.0 mg/l, for the 100 ppm treatment group. The residue concentrations among treatment groups were significantly different ($p<0.05$) in the muscle. A dose response relationship was apparent (Figure 10). The plasma, however, only had single samples analyzed therefore statistical analysis was not possible, but a dose response relationship was suggested (Figure 11). Aroclor 1254 residues were higher in the skin as compared to the other tissues (Table 8). Skin from the control birds contained 21.0 ppm, and the 100 ppm group had 2295 ppm of Aroclor 1254 residues (Table 8). Treatments were significantly different ($p<0.05$) and a dose response relationship was evident (Figure 12).

The budgie diet had Aroclor 1254 residues measuring less than 50% of the calculated dietary concentration based on the amounts weighed out and blended into the diet. As shown in Table 9, the control group had 1.4 ppm Aroclor 1254, and the 50 and 100 ppm groups had only 15.4 and 48.7 ppm Aroclor 1254, respectively.

Body weights of budgies were not affected by the dietary Aroclor 1254 in Experiment II (Table 10). The control and 100 ppm group had an average body weight of 34.8 g and 35.1 g, respectively, at the onset of the study (Table 10). The net change from day 0 to day 28 was not significant ($p>0.05$)

TABLE 8 RESIDUAL AROCLOR 1254 CONCENTRATIONS IN TISSUES FROM BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254 (ppm)	ORGAN	AROCLOR 1254 RESIDUE* (ug/g tissue)
	MUSCLE	
0.0		0.5±0.15
12.0		1.7±0.0
25.0		3.6±0.5
50.0		7.1±1.71
100.0		12.5±2.3
	SKIN	
0.0		21.1±11.2
12.5		209.1±20.3
25.0		182.5±11.9
50.0		409.4±231.2
100.0		2295.0±405.8
	PLASMA	
0.0		0.4±0.1
12.0		1.5±0.0˜
25.0		2.7±0.0
50.0		2.3±0.0
100.0		10.0±0.0

*values represent mean±std.dev.
-sample size is 5 birds/treatment
˜plasma contains single values due to limited sample size

TABLE 9 NOMINAL AND ACTUAL AROCLOR 1254 CONCENTRATIONS IN THE DIETS USED IN EXPERIMENTS I AND II

NOMINAL AROCLOR 1254 (ppm)	ACTUAL AROCLOR	ACTUAL AROCLOR
	EXPT. I	EXPT. II
0.0 ppm	1.4	0.0
12.5 ppm	5.2	5.1
25.0 ppm	9.9	7.0
50.0 ppm	15.4	17.4
100.0 ppm	48.7	40.5

TABLE 10 BUDGIE WEIGHTS FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS IN EXPERIMENT II

DIETARY AROCLOR 1254 (ppm)	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	NET CHANGE (g)~
0.0	34.8±2.5	36.0±2.6	1.22±2.6
12.5	35.4±2.1	35.9±3.0	0.54±3.0
25.0	33.2±2.8	35.4±3.1	1.51±3.6
50.0	34.0±3.6	34.3±4.1	-1.36±5.4
100.0	35.1±2.8	34.4±3.0	2.83±4.2

*values represent mean±std. dev.; sample size was 10/group
~initial-final=net change

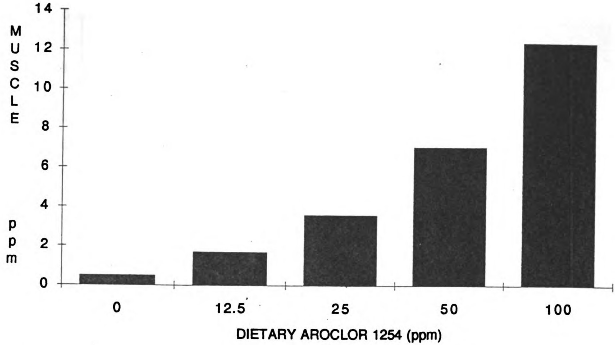

FIGURE 10 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE MUSCLE FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

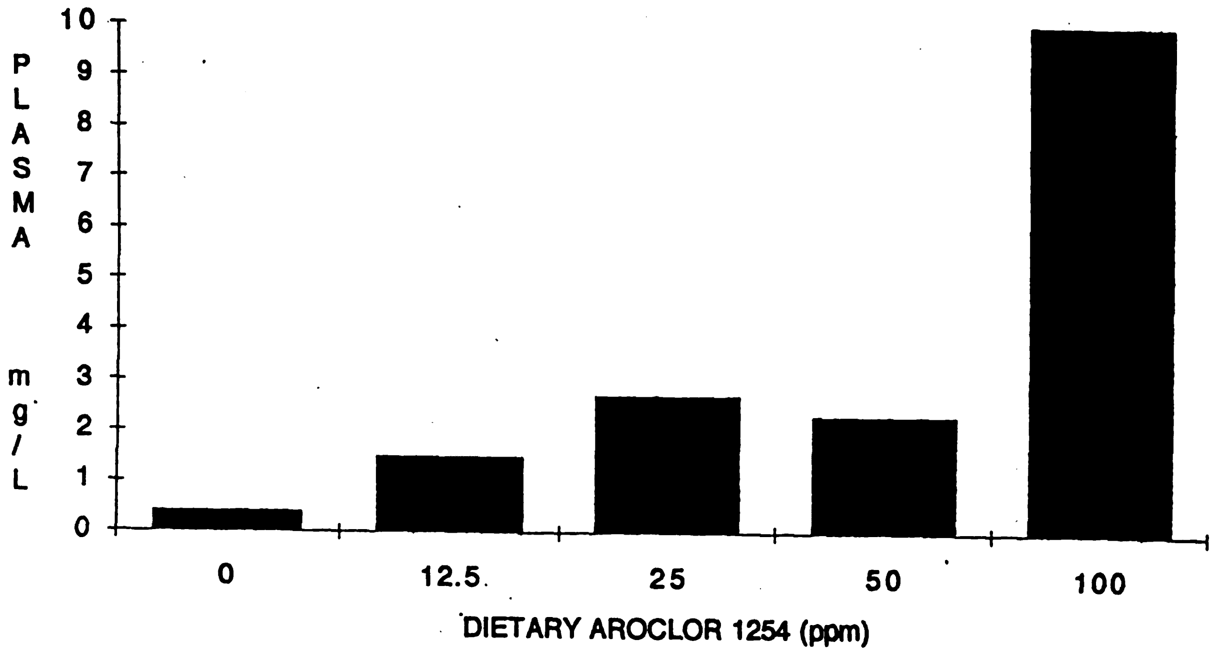

FIGURE 11 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE PLASMA FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

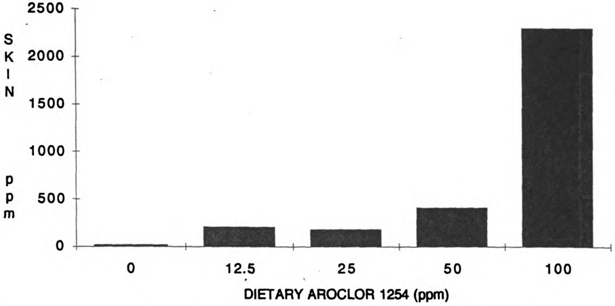

FIGURE 12 RESIDUAL AROCLOR 1254 CONCENTRATIONS MEASURED IN BUDGIE SKIN FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

between any of the treatments. Feed consumption in experiment II was 9.33 g/b/d for the control group and 8.43 g/b/d for the 100 ppm group (Table 11). The lowest amount consumed was 7.48 g/b/d by the 50 ppm group. A statistical analysis for feed consumption was not possible since measurements were taken on day 0 and day 28 resulting in one value. Mortality in experiments I and II were 4% and 10%, respectively. There was no significance between the death of the bird and the treatment group it was in.

Microsomal protein concentration of budgie livers averaged 1.07 mg/g liver in the control group and 2.82 mg/g in the 100 ppm group (Table 12). There were no significant differences between groups ($p>0.05$) however, a dose response relationship was indicated.

The 7-ethoxyresorufin activity measured in the control birds averaged 0.29 mg resorufin/mg protein/minute (Table 13). The treated groups had significantly higher ($p<0.05$) values than the control. The 25 ppm group had the lowest value, (47 mg/mg/min) whereas the 12.5 ppm group had the highest value, (72.2 mg/mg/min) (Table 13).

Figure 13 is a typical Aroclor 1254 standard illustrating the 6 major peaks used for comparison; peak #1 (40 mm from point of injection), #2 (49 mm), #3 (60 mm), #4 (72 mm), #5 (84 mm), and #6 (100 mm). Tables 14 and 15 contain the same data but compare different parameters. Table 14 represents the amount (%) that each of the 6 major peaks of the Aroclor

TABLE 11 FEED CONSUMPTION OF BUDGIES FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS IN EXPERIMENT II*

DIETARY AROCLOR 1254				
0.0 ppm	**12.5 ppm**	**25.0 ppm**	**50.0 ppm**	**100.0 ppm**
9.33**	8.39	8.25	7.48	8.43

*grams of feed consumed/bird/day
**feed intake was determined at the end of the experiment therefore only one value was available
- values are expressed as mean±standard deviation;
- sample size was 10/group

TABLE 12 HEPATIC MICROSOMAL CONCENTRATIONS IN BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254 (ppm)	MICROSOMAL PROTEIN CONC. mg/g LIVER
0.0	1.07±0.4*
12.5	2.26±0.9
25.0	2.33±0.8
50.0	3.13±1.7
100.0	2.82±1.8

*values represent mean±std.dev.
-sample size is 6 in the 0.0 ppm group and 3 in the treated groups

TABLE 13 7-ETHOXYRESORUFIN-O-DEETHYLASE (EROD) ACTIVITY IN BUDGIES EXPOSED TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254 (ppm)	EROD ACTIVITY mg resorufin/mg protein/min
0.0	0.29±1.8*
12.5	72.2±28.8
25.0	47.0±4.5
50.0	71.6±13.5
100.0	62.3±26.8

*values represent mean±std.dev.
-treatment groups are significantly higher than the 0.0 ppm group at $p \leq 0.05$

TABLE 14 THE AMOUNT (%) THAT EACH OF THE SIX MAJOR PEAKS OF THE AROCLOR 1254 STANDARD CONTRIBUTE TO THE TOTAL RESIDUE IN TISSUE

PEAK #	1-40mm	2-49mm	3-60mm	4-72mm	5-84mm	6-100mm
			PERCENT			
AROCLOR 1254	15.4	8.3	11.3	30.0	23.0	12.1
			0.0 ppm			
CARCASS	12.1	17.0	16.2	22.2	24.1	8.3
MUSCLE	6.7	14.8	15.9	24.3	27.2	11.9
BRAIN	8.5	34.7	2.1	39.9	14.8	0.0
LIVER	4.5	25.8	12.2	31.7	22.8	3.1
SKIN	7.5	10.1	11.3	29.7	23.9	17.6
			12.5ppm			
CARCASS	4.2	8.1	12.3	33.8	20.2	21.3
MUSCLE	5.1	10.1	13.6	30.9	23.0	17.0
BRAIN	0.0	28.5	0.0	29.2	31.5	10.9
LIVER	3.1	13.2	3.1	38.4	29.0	13.1
SKIN	4.9	9.6	13.8	29.6	21.7	20.3
			25 ppm			
CARCASS	3.8	12.4	11.5	30.8	23.1	18.6
MUSCLE	4.1	12.7	12.2	29.4	23.2	18.6
BRAIN	0.0	17.9	0.0	39.6	32.0	10.5
LIVER	3.1	11.5	3.4	36.7	27.6	17.8
SKIN	3.7	11.6	13.6	29.4	22.4	19.3

TABLE 14 (cont'd.)

PEAK #	1-40mm	2-49mm	3-60mm	4-72mm	5-84mm	6-100mm
			PERCENT			
AROCLOR 1254	15.4	8.3	11.3	30.0	23.0	12.1
			50 ppm			
CARCASS	3.6	12.3	11.3	29.7	23.6	19.6
MUSCLE	2.7	13.6	11.2	27.6	25.1	19.7
BRAIN	2.7	12.8	9.0	35.7	30.6	9.2
LIVER	2.3	10.3	5.8	35.4	27.0	19.2
SKIN	2.6	10.2	10.3	30.3	24.2	22.5
			100 ppm			
CARCASS	3.1	11.5	11.9	30.3	22.9	20.3
MUSCLE	3.2	12.1	13.3	28.3	19.7	19.9
BRAIN	2.7	14.9	7.5	33.0	30.3	11.6
LIVER	2.9	10.5	5.4	36.2	27.2	17.8
SKIN	2.7	11.3	12.2	30.0	23.1	20.8

TABLE 15 GC ANALYSIS OF WHOLE ORGANS AND TISSUES OF BUDGIES⁻ FOLLOWING EXPOSURE TO DIETARY AROCLOR 1254 FOR 28 DAYS

DIETARY AROCLOR 1254 (ppm)

PEAK NO. (mm)	AROCLOR 1254 STD.	CARCASS				
		0.0	12.5	25.0	50.0	100.0
1-40	15.4	12.1c*	4.2b	3.8ab	3.6ab	3.1a
2-49	8.3	17.0c	8.1b	12.4a	12.3a	11.5a
3-60	11.3	16.2c	12.3a	11.5a	11.3a	11.9a
4-72	30.0	22.2c	33.8a	30.8b	29.7b	30.3b
5-84	23.0	24.1a	20.2a	23.1a	23.6a	22.9a
6-100	12.1	8.3b	21.3a	18.6a	19.6a	20.3a
		MUSCLE				
1-40	15.4	6.7d	5.1a	4.1ac	2.7b	3.2bc
2-49	8.3	14.8a	10.1a	12.7a	13.6a	12.1a
3-60	11.3	15.9a	13.6a	12.2a	11.2a	13.3a
4-72	30.0	24.3c	30.9a	29.4ab	27.6b	28.3b
5-84	23.0	27.2a	23.0a	23.2a	25.1a	19.7a
6-100	12.1	11.9a	17.4a	18.6a	19.7a	19.9a
		BRAIN				
1-40	15.4	8.5c	0.0a	0.0a	2.7b	2.7b
2-49	8.3	34.7a	28.5a	17.9ab	12.8b	14.9b
3-60	11.3	2.1a	0.0a	0.0a	9.0b	7.5b
4-72	30.0	39.9c	29.2a	39.6c	35.7b	33.0ab
5-84	23.0	14.8b	31.5a	32.0a	30.6a	30.3a
6-100	12.1	0.0b	10.9a	10.5a	9.2a	11.6a

* values having the same letter are not significantly different $p>0.05$

⁻ values are the percent of the individual peak height,(mm), per sum of the total peaks analyzed

TABLE 15 (cont'd.)

PEAK NO. (mm)	AROCLOR 1254 STD.	DIETARY AROCLOR 1254 (ppm)				
		LIVER				
		0.0	12.5	25.0	50.0	100.0
1-40	15.4	4.5a*	3.1a	3.1a	2.3a	2.9a
2-49	8.3	25.8b	13.2b	11.5a	10.3a	10.5a
3-60	11.3	12.2b	3.1a	3.4a	5.8a	5.4a
4-72	30.1	31.7a	38.4a	36.7a	35.4a	36.2a
5-84	23.0	22.8a	29.0a	27.6a	27.0a	27.2a
6-100	12.1	3.1c	13.1a	17.8b	19.2b	17.8b
		SKIN				
1-40	15.4	7.5c	4.9a	3.7ab	2.6b	2.7b
2-49	8.3	10.1a	9.6a	11.6a	10.2a	11.3a
3-60	11.3	11.3a	13.8a	13.6a	10.3a	12.2a
4-72	30.1	29.7a	29.6a	29.4a	30.3a	30.0a
5-84	23.0	23.9a	21.7a	22.4a	24.2a	23.1a
6-100	12.1	17.6a	20.3a	19.3a	22.5a	20.8a

* values having the same letter are not significantly different p>0.05

~ values are the percent of the individual peak height,(mm), per sum of the total peaks analyzed

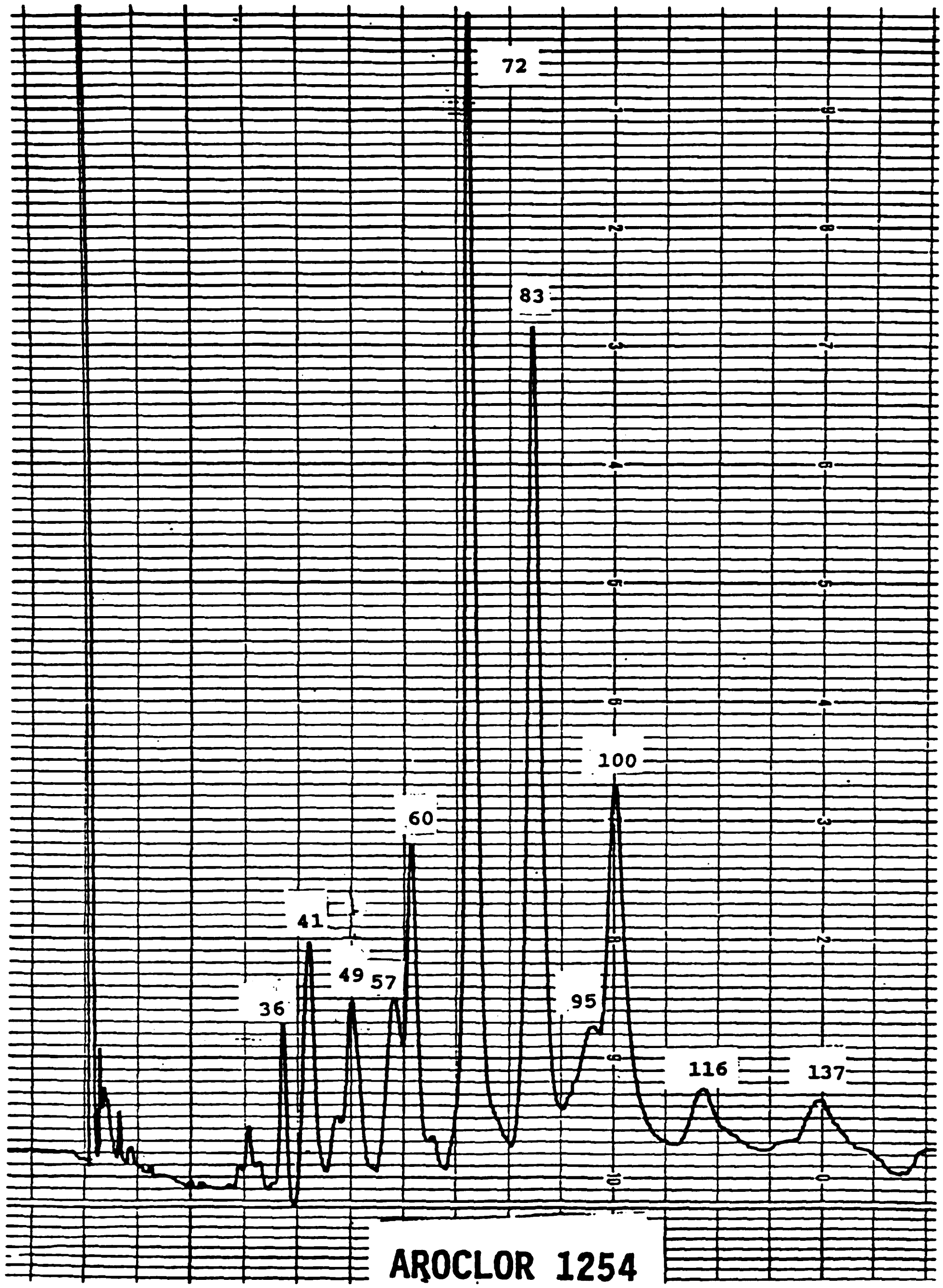

FIGURE 13 GAS CHROMATOGRAPHY ANALYSIS OF AROCLOR 1254 STANDARD

1254 standard contributes to the total residue in the tissues. Table 15 compares the tissues with the different treatment concentrations. In comparing the percentage of each congener of the standard to the test solution (Table 14), all tissues have a lower percentage of congener residue than the standard in peak #1. The brain has the lower percentage of congener residue in peaks #3 and #6. All tissues contain a similar percentage of congener residue as compared to the standard in peaks #2, #4 and #5. As shown in Table 15, the carcass has inconsistent peak percentages among treatments. The muscle and skin, on the other hand, have fairly consistent peak percentages among treatments. Finally, the brain and liver have various peak values among treatments.

DISCUSSION

BODY WEIGHTS, FEED CONSUMPTION, AND MORTALITY

Aroclor 1254 fed to budgies at concentrations ranging from 12.5 to 100 ppm for 28 days did not cause any adverse effects in body weight, feed consumption, or mortality. The lack of an effect on body weight reported here is in agreement with Prestt et al. (1970) who found no effect on the body weights of Bengalese finches when they were fed up to 400 ppm Aroclor 1254 for 56 days. Laying hens fed up to 20 ppm Aroclor 1248 show no effect on feed consumption (Scott et al., 1971). The low percentage of mortality occurring from feeding Aroclor 1254 to budgies did not appear to be due to the xenobiotic. Tucker and Crabtree (1970) fed chickens up to 2000 ppm of either Aroclor 1242, 1254, 1260 or 1268 and reported no mortality. Prestt et al. (1970) reported that dietary Aroclor 1254 had a 5 day LD_{50}=1090 mg/kg body weight of pheasants.

RELATIVE ORGAN WEIGHTS

The significant increase in relative liver weights at the higher dose group is in agreement with Vos and Koeman, (1970) who fed chicks 400 ppm Aroclor 1260 for 60 days. Grant et al. (1971) reported similar results in studies with male Wistar rats. The oral administration of 500 mg/kg significantly increased the size of the rat's liver.

RESIDUES IN TISSUES

The skin contained the highest concentration of Aroclor 1254 followed by the liver, kidney, brain, muscle and plasma, in that order. Breslin et al. (1983) reported a similar pattern for the accumulation of hexachlorobenzene in female bobwhite tissues. Adipose tissue contained the highest concentration followed by skin, liver, brain, heart and kidney, whole blood and muscle. The adipose appears to be the primary tissue to accumulate the highest level of Aroclor 1254 residues; whereas the blood and muscle tissue appear to contain the lowest concentration of Aroclor 1254 (Grant et al., 1971). This was similar for the budgies. If adipose tissue had been analyzed in the present study, it may have contained the highest level of Aroclor 1254. This parameter was not considered knowing that budgies are very lean birds. The muscle tissue of budgies and the blood contain few lipids therefore it was not unexpected that they would contain low levels of Aroclor 1254 residues.

Absorption of topically administered PCBs has been studied by Wester et al. (1983) in guinea pigs which were dermally exposed to 50 ul of a ^{14}C-labeled 54% PCB solution containing 5.2 uCi. Their results indicated that 56% of the topical dose was absorbed. Budgies will habitually roll in their feed cup only to get fully covered with the dust from their diet; in this case, Aroclor 1254 diet. This might explain the high residue of Aroclor 1254 in the defeathered skin. If dermal absorption occurs readily with topical administration, as Wester et al. (1983) has shown, then some dermal absorption of Aroclor 1254 contaminated diet may contribute to the high residues in the budgie skin. Residues of Aroclor 1254 in the liver of budgies is expected to be high. This organ is primarily responsible for metabolism of the congeners that results in less toxicity of parent compound and enhances the elimination of the metabolite. The kidney usually has lower concentrations than the liver since its role is primarily excretion of the changed compound. Finally, the brain will be either equivalent to the kidney or contain lower residue levels. In agreement with Dahlgren et al. (1971), Aroclor 1254 residues accumulated in the pheasant brain due to its high lipid content, as they did in the budgies. Since the carcass is a mixture of all organs and tissues, it will reflect the sum of the Aroclor 1254 in the tissues.

Polin et al. (1986) fed 10 ppm hexachlorobenzene (HCB) to young chicks for 14 days. The average body burden was 573 ug of HCB or 8% of the total amount of HCB administered. Dahlgren et al. (1971) reported that 50 mg doses of Aroclor 1254 given to pheasants once a week for 17 weeks resulted in carcass residues of 60 to 82% of the total amount of PCBs administered. The budgie body burdens for the 100 ppm group were only 1.5% of the total amount of Aroclor 1254 administered for 28 days.

PERCENT PEAK VALUES

The Aroclor 1254 standards used in the GC analysis ranged from 0.2365 to 2.365 ppm. The peak heights of the 6 major peaks of the standards were compared to the corresponding peak heights of the test solutions. Bagley et al. (1970) have investigated the individual peaks of the Aroclor 1254 standard using a gas-liquid chromatography-mass spectrometry (GLC-MS). In comparison to the peaks shown in Figure 13, peaks 1 and 2 will represent congeners containing 4 chlorine atoms and a molecular weight of 290. Peaks 3,4, and 5 contain 5 chlorine atoms and 324 molecular weight and peak 6 has 6 chlorine atoms and 358 molecular weight. The patterns illustrated by the tissue residues in Table 14 indicate that each tissue represents a different percentage of individual congeners. Different factors that may contribute to this variability include lipid content in the tissue, rate of blood flow to the tissue, and the primary role of the tissue. In Table 15, the carcass' inconsistent peak percentages among the treatments may be due to its mixture of all tissues. The muscle, on the other hand, has fairly consistent peak percentages among treatments. This is clearly due to the lack of lipid content in the muscle and the high rate of blood flow to the organ. The different values shown by the brain residue suggests that this organ may be shifting the compound to make it more soluble in an attempt to metabolize the xenobiotic. The liver also illustrated various peak values. Its primary role is to metabolize the congeners so it is not surprising to observe these various percentages. Finally, the skin, which is a storage site for Aroclor 1254, had similar peak percentages because it will store the compound without changing the chlorine atoms of the molecule.

MICROSOMAL PROTEIN CONCENTRATION AND EROD ACTIVITY

Statistical elevation of microsomal protein concentration among treatments did not occur in the budgie experiment. This is in agreement with Cecil et al. (1978) who fed 5, 50, and 500 ppm Aroclor 1254 to White Leghorn chickens. 7-ethoxyresorufin deethylase activity was significantly higher in the treated budgie livers when compared to the control livers. Similar studies by Rifkind et al. (1984) showed that injection of 5, 50, 500, and 5000 nmol hexachlorobenzene/egg induced 7-ethoxyresorufin in all dose levels.

CONSIDERATIONS FOR FUTURE RESEARCH

If future research is to be conducted on parakeets pertaining to Aroclor residues and enzyme induction, a pilot study to establish an LC_{50} should be performed. In comparing individual housing and multiple birds per cage, an individual cage system would be ideal since these birds have a defined social structure, and social behavior may influence feed consumption or mortality. Finally, dietary samples should be analyzed following a procedure specific for the dietary ingredients.

CONCLUSION

PCBs have been increasingly identified as a significant environmental pollutant. Exposure of humans to PCBs is possible either directly or indirectly. PCBs may directly enter the food chain through accidental contamination. They may indirectly get into the food chain through their use in packaging material which may contaminate the food. Finally, PCBs may enter the animal food chain through fish being contaminated or food producing animals being contaminated, and their carcasses incorporated into the feed. The FDA has set tolerance limits for such food products as listed in the Introduction. The purpose of this study may have been indirectly related to humans; yet, it has given the researchers additional information relative to the contaminant and the effects experienced by the budgie.

The purpose of this study was: 1) To determine if Aroclor 1254, fed at high concentrations in the diet, is toxic to the American budgerigar, 2) To determine the residues in selected tissues from feeding Aroclor 1254 to the American budgerigar and 3) To determine the activity of hepatic 7-ethoxyresorufin-O-deethylase in budgie livers as an indicator for detoxification mechanisms. The experimental design was developed such that the budgies were fed different dietary concentrations of Aroclor 1254 for 28 days. In experiment I, tissue residues were determined. In experiment II, the effect of different levels of Aroclor 1254 on liver enzyme induction was evaluated.

Residue analysis determined that Aroclor 1254 concentrations were highest in the skin, followed by the liver, kidney, brain, muscle and plasma. The liver, kidney and brain organ weights were measured on a percent organ/body weight. Results indicated the liver weight was significantly higher ($p<0.05$) for budgies fed 50 and 100 ppm Aroclor 1254 as compared to the control group. The kidney and brain organ weights were not significantly different among treatments. Furthermore, the data indicated that short term feeding of Aroclor 1254 to budgies did not result in mortality or clinical signs.

Hepatic microsomal protein concentrations were not statistically significant among treatment groups; yet, a dose response relationship was apparent. Maximal induction of 7-ethoxyresorufin-O-deethylase occurred at the lowest dietary Aroclor 1254 concentration; 12.5 ppm.

APPENDIX

APPENDIX A

TABLE A BODY WEIGHTS OF BUDGIES IN EXPT. I

DIETARY AROCLOR 1254	INITIAL WEIGHT (grams)	FINAL WEIGHT (grams)	NET CHANGE (grams)
0.0 ppm	35.5	34.0	-1.5
	34.5	34.6	0.1
	32.0	32.8	0.8
	30.5	28.8	-1.7
	31.5	28.2	-3.3
	34.5	32.6	-1.9
	32.5	32.2	-0.3
	31.5	31.2	-0.3
	33.5	34.8	1.3
mean ± std.dev.	32.9±1.6	32.1±2.2	
12.5 ppm	31.5	24.3	-7.2
	32.5	32.7	0.2
	34.5	35.0	0.5
	33.5	34.2	0.7
	30.5	30.2	-0.3
	31.0	28.3	-2.7
	36.5	35.8	-0.7
	32.5	33.5	1.0
	30.0	32.8	2.8
mean ± std.dev.	32.5±2.0	31.9±3.5	
25.0 ppm	30.5	30.0	-0.5
	31.5	30.5	-1.0
	31.5	33.3	1.8
	34.5	25.3	-9.2
	28.0	29.6	1.6
	33.0	34.0	1.0
	33.0	32.0	-1.0
	32.0	31.5	-0.5
	35.0	37.4	2.4
	28.5	28.5	0.0
mean ± std.dev.	31.8±2.2	31.2±3.1	

APPENDIX A
TABLE A (cont'd.)

DIETARY AROCLOR 1254	INITIAL WEIGHT (grams)	FINAL WEIGHT (grams)	NET CHANGE (grams)
50.0 ppm	35.0	33.3	-1.7
	32.0	NA*	-
	30.5	28.0	-1.5
	34.5	29.0	-5.5
	34.5	38.0	3.5
	35.0	36.5	1.5
	34.0	33.5	-0.5
	31.0	29.5	-1.5
	30.5	30.8	-0.3
	27.0	NA*	-
mean ± std.dev.	32.4±1.8	32.3±3.4	
100.0 ppm	34.5	36.0	1.5
	33.5	34.5	1.0
	29.0	NA*	-
	35.0	35.3	0.3
	32.5	32.0	-0.5
	31.0	35.5	4.5
	32.5	34.0	1.5
	30.0	32.8	2.8
	33.5	34.6	1.1
	32.5	36.6	4.1
mean ± std.dev.	32.4±1.8	34.6±1.4	

*NA=bird not alive on specified day

APPENDIX B

TABLE B FEED CONSUMPTION OF BUDGIES IN EXPERIMENT I*

DIETARY AROCLOR 1254	WEEK 1	WEEK 2	WEEK 3	WEEK 4	MEAN ± STD.DEV.
0.0 ppm	10.4	7.2	9.0	6.5	8.3±1.8
12.5 ppm	10.2	6.0	8.6	5.5	7.6±2.2
25.0 ppm	6.9	6.9	7.6	5.6	6.8±2.4
50.0 ppm	10.0	9.1	5.7	5.1	7.5±2.4
100.0 ppm	8.4	9.0	6.2	6.5	7.5±1.4

*feed consumption is measured in gram/bird/day; 10 birds/group

TABLE B1 FEED CONSUMPTION OF BUDGIES IN EXPERIMENT II*

DIETARY AROCLOR 1254	FINAL WT.-INITIAL WT. (grams)
0.0 ppm	9.3
12.5 ppm	8.4
25.0 ppm	8.3
50.0 ppm	7.5
100.0 ppm	8.4

*one value for feed intake was obtained therefore a statistical analysis was not possible

APPENDIX C

TABLE C WHOLE ORGAN WEIGHTS (g)

DIETARY AROCLOR 1254	LIVER					MEAN ± STD.DEV.
0.0 ppm	0.4	0.6	0.7	0.6	0.5	0.57±1.0
12.5 ppm	0.5	0.6	0.7	0.6	0.8	0.64±1.0
25.0 ppm	0.7	0.9	0.7	0.7	0.6	0.71±1.0
50.0 ppm	0.9	0.8	1.0	0.7	1.5	0.98±0.27
100.0 ppm	0.9	0.6	1.0	1.63*	0.9	1.03±0.33
	KIDNEY					
0.0 ppm	0.3	0.3	0.3	0.2	0.2	0.27±0.03
12.5 ppm	0.2	0.3	0.3	0.3	0.3	0.29±0.05
25.0 ppm	0.3	0.2	0.2	0.3	0.2	0.24±0.04
50.0 ppm	0.2	0.3	0.3	0.2	0.3	0.25±0.02
100.0 ppm	0.3	0.3	0.3	0.4	0.3	0.33±0.03
	BRAIN					
0.0 ppm	1.3	0.7	1.1	1.1	1.2	1.07±0.18
12.5 ppm	1.2	1.2	0.9	1.0	1.0	1.06±0.12
25.0 ppm	1.2	1.2	1.1	1.1	1.0	1.12±0.07
50.0 ppm	1.3	1.1	0.9	1.2	1.1	1.13±0.13
100.0 ppm	1.2	1.2	1.1	1.1	1.2	1.17±0.06

*a growth or tumor was present on this liver

APPENDIX D

TABLE D SAMPLE WEIGHTS OF TISSUES (g) FOR GAS CHROMATOGRAPHY ANALYSIS OF AROCLOR 1254

DIETARY AROCLOR 1254 (ppm)	0.0	12.5	25.0	50.0	100.0
CARCASS	7.0	10.0	10.0	10.1	10.0
	4.6	10.0	10.3	10.8	10.5
	3.9	14.4	11.6	10.1	10.9
	3.7	15.8	10.8	10.3	10.5
	3.9	10.0	10.5	10.3	10.3
		10.0	11.2	11.0	10.1
		10.1	10.5	10.5	10.1
		10.2	10.2	10.0	10.1
		9.7	10.1	10.0	10.0
		10.0	13.0	10.1	10.1
LIVER	0.9	1.1	1.3	1.7	1.9
	0.8	0.9	1.0	2.0	1.9
KIDNEY	0.8	0.8	0.6	0.8	0.9
BRAIN	1.7	2.0	2.3	1.9	2.3
	1.7	1.8	2.3	2.0	2.3
MUSCLE	10.8	13.3	10.5	10.8	14.9
	16.1	15.7	12.4	17.0	19.0
SKIN	0.8	1.1	0.7	0.9	1.1
	1.0	1.3	0.7	0.7	0.9
PLASMA	3.0	2.2	1.4	2.6	2.2
	2.0				

APPENDIX E

TABLE E BODY WEIGHTS OF BUDGIES IN EXPERIMENT II (g)

DIETARY AROCLOR 1254	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	NET CHANGE
0.0 ppm	33.3	35.8	38.7	35.3	33.4	0.1
	39.0	41.3	42.7	43.8	39.6	0.6
	37.6	37.7	38.8	35.9	38.6	1.0
	34.6	29.5	30.3	35.3	35.6	1.0
	36.0	36.3	35.7	36.5	37.1	1.1
	33.3	35.4	34.7	38.2	37.0	3.7
	31.3	31.5	31.7	31.5	30.5	-0.8
	32.3	32.1	28.8	35.6	35.1	2.8
	35.7	39.1	36.3	36.2	37.3	1.6
mean±s.d	34.8±3	35.4±4	35.3±4	36.5±3	36.0±3	
12.5 ppm	33.9	34.1	37.3	36.5	34.5	0.6
	35.0	35.8	38.3	37.5	37.4	2.4
	35.3	35.8	36.8	37.4	35.5	0.2
	36.5	35.3	36.5	37.5	36.1	-0.4
	39.3	35.9	37.7	39.3	41.9	2.6
	32.3	33.1	31.7	32.8	31.0	-1.3
	37.8	37.7	40.0	39.5	38.5	0.7
	35.1	36.4	40.1	39.1	37.3	2.2
	36.1	36.5	36.7	38.5	35.1	-1.0
	33.0	30.4	31.3	32.6	32.1	-0.9
mean±s.d	35.4±2	35.1±2	36.6±3	37.1±2	35.9±3	

APPENDIX E

TABLE E (cont'd).

DIETARY AROCLOR 1254	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	NET CHANGE
25.0 ppm	33.8	31.2	36.7	36.8	37.5	3.7
	32.4	33.5	35.8	35.2	34.0	1.6
	33.5	33.4	35.3	40.4	35.7	2.2
	35.5	38.2	39.4	41.2	39.0	3.5
	37.0	37.6	40.7	39.3	35.9	-1.1
	30.8	30.2	31.6	28.3	NA*	-
	31.5	31.2	33.3	36.5	35.2	3.7
	34.5	34.5	36.6	36.8	36.9	2.4
	27.4	25.4	29.3	28.8	27.6	0.2
	35.5	37.5	40.4	39.0	37.0	1.5
mean$\pm$s.d	33.2$\pm$3	33.3$\pm$4	35.9$\pm$4	36.2$\pm$4	35.4$\pm$3	
50.0 ppm	31.5	32.4	32.3	31.3	26.5	-5.0
	35.8	38.3	39.5	37.1	35.8	0.0
	42.7	41.2	42.5	40.7	42.3	-0.4
	33.7	34.2	35.7	35.3	33.2	-0.5
	30.5	30.4	29.7	31.0	32.5	2.0
	34.1	34.8	32.4	35.3	33.7	-0.4
	35.9	32.5	21.0	NA	-	-
	30.2	32.2	31.0	NA	-	-
	32.5	35.8	35.4	35.8	35.2	2.7
	32.8	33.3	35.6	36.0	35.2	2.4
mean$\pm$s.d	34.0$\pm$4	34.4$\pm$3	34.9$\pm$4	35.3$\pm$3	34.3$\pm$4	

*NA=bird not alive on specified week

APPENDIX E

TABLE E (cont'd).

DIETARY AROCLOR 1254	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	NET CHANGE
100 ppm	36.3	35.6	35.5	37.7	31.2	-3.8
	35.2	39.5	41.5	40.8	37.4	2.2
	33.4	33.5	34.0	35.5	35.0	1.6
	37.4	38.8	34.7	33.2	30.5	-6.9
	32.5	32.9	34.5	30.9	31.5	-1.0
	38.2	33.8	28.2	24.8	NA*	-
	37.6	34.5	38.5	37.7	34.0	-3.6
	30.6	39.4	42.6	43.5	39.7	9.1
	31.7	31.5	34.3	34.0	NA	-
	37.6	37.9	38.7	28.9	NA	-
mean±s.d	35.1±3	35.7±3	36.3±4	35.8±4	34.4±3	

*NA=bird not alive on specified week

APPENDIX F

TABLE F MICROSOMAL PROTEIN CONCENTRATIONS IN BUDGIES (mg/L)

DIETARY AROCLOR 1254	0.0 ppm	12.5 ppm	25.0 ppm	50.0 ppm	100.0 ppm
	1.1	3.6	3.4	5.6	5.2
	0.8	1.7	1.7	1.8	1.1
	1.7	1.5	1.9	2.1	2.2
	0.9				
	0.8				
mean ± s.d	1.07±0.4	2.26±0.9	2.33±0.8	3.13±0.8	2.82±1.8

APPENDIX G

TABLE G **7-ETHOXYRESORUFIN-O-DEETHYLASE (EROD) CONCENTRATION IN BUDGIES***

DIETARY AROCLOR 1254	0.0 ppm	12.5 ppm	25.0 ppm	50.0 ppm	100.0 ppm
	0.4	54.2	40.8	88.6	95.5
	0.0	112.8	51.4	70.5	29.9
	0.4	49.7	48.7	55.6	61.5
mean±s.d	0.29±0.2	72.2±29.0	47.0±4.5	71.6±13.5	62.3±26.8

*data represents mg resorufin/mg protein/minute

APPENDIX H

TABLE H1 STATISTICAL ANALYSIS

DIETARY AROCLOR 1254 VS. BODY WEIGHT CHANGE IN 28 DAYS FOR EXPERIMENT I

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	9.0	-0.8	1.4	0.5
12.5 ppm	9.0	-0.6	2.9	1.0
25.0 ppm	10.0	-0.5	3.3	1.0
50.0 ppm	10.0	-1.5	3.6	1.1
100.0 ppm	10.0	1.1	2.7	0.8
		ANOVA		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	36.9	9.2	1.1
WITHIN GROUPS	43.0	360.7	8.4	P=0.3687
TOTAL	47.0	397.6		

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=body weight change (g)

APPENDIX H

TABLE H2 STATISTICAL ANALYSIS

DIETARY AROCLOR 1254 VS. BODY WEIGHT CHANGE
IN 28 DAYS FOR EXPERIMENT II

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	9.0	1.2	1.4	0.5
12.5 ppm	10.0	0.5	1.5	0.5
25.0 ppm	9.0	2.0	1.6	0.5
50.0 ppm	8.0	0.1	2.5	0.9
100.0 ppm	7.0	1.6	5.0	1.9
		ANOVA		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	20.3	5.1	0.8
WITHIN GROUPS	38.0	245.6	6.5	P=0.5414
TOTAL	42.0	265.9		

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=body weight change (g)

APPENDIX H

TABLE H3 STATISTICAL ANALYSIS

THE EFFECT OF DIETARY AROCLOR 1254 ON % LIVER/BODY WEIGHT

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	5.0	1.8	0.4	0.2
12.5 ppm	5.0	2.0	0.4	0.2
25.0 ppm	5.0	2.3	0.3	0.1
50.0 ppm	5.0	2.9	0.8	0.4
100.0 ppm	5.0	3.0	1.2	0.5
		ANOVA		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	6.3	1.6	3.2
WITHIN GROUPS	20.0	9.8	0.5	P=0.034
TOTAL	24.0	16.1		

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=% organ/body wt

APPENDIX H

TABLE H4 STATISTICAL ANALYSIS

THE EFFECT OF DIETARY AROCLOR 1254 ON % KIDNEY/BODY WEIGHT

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	5.0	0.8	0.1	0.0
12.5 ppm	5.0	0.9	0.1	0.1
25.0 ppm	5.0	0.8	0.1	0.1
50.0 ppm	5.0	0.8	0.1	0.1
100.0 ppm	5.0	1.0	0.1	0.1
		ANOVA		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	0.1	0.0	1.9
WITHIN GROUPS	20.0	0.3	0.0	P=0.1482
TOTAL	24.0	0.5		

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=% organ/body wt

APPENDIX H

TABLE H5 STATISTICAL ANALYSIS

THE EFFECT OF DIETARY AROCLOR 1254 ON % BRAIN/BODY WEIGHT

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	5.0	3.3	0.7	0.3
12.5 ppm	5.0	3.4	0.7	0.3
25.0 ppm	5.0	3.7	0.3	0.1
50.0 ppm	5.0	3.4	0.4	0.2
100.0 ppm	5.0	3.5	0.2	0.1
		ANOVA		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	0.4	0.1	0.4
WITHIN GROUPS	20.0	4.8	0.2	P=0.8131
TOTAL	24.0	5.2		

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=% organ/body wt

APPENDIX H

TABLE H6 STATISTICAL ANALYSIS

THE EFFECT OF DIETARY AROCLOR 1254
ON MICROSOMAL PROTEIN IN BUDGIES

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	5.0	1.1	0.4	0.2
12.5 ppm	3.0	2.3	1.1	0.7
25.0 ppm	3.0	2.3	0.9	0.5
50.0 ppm	3.0	3.1	2.1	1.2
100.0 ppm	3.0	2.8	2.2	1.2
		ANOVA*		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	10.3	2.6	1.3
WITHIN GROUPS	12.0	23.0	1.9	P=0.3112
TOTAL	16.0	33.3		
COMPARISON	MEAN DIFF.	FISHER PLSD	SCHEFFE F-TEST	DUNNETT T-TEST
0.0 : 12.5	-1.192	2.203	0.348	1.179
0.0 : 25.0	-1.265	2.203	0.392	1.252
0.0 : 50.0	-2.069	2.203	1.047	2.046
0.0 : 100	-1.759	2.203	0.757	1.740

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=protein mg/ml

APPENDIX H (cont'd)

TABLE H6

COMPARISON	MEAN DIFF.	FISHER PLSD	SCHEFFE F-TEST	DUNNETT T-TEST
12.5 : 25	-0.073	2.463	0.00105	0.065
12.5 : 50	-0.877	2.463	0.150	0.766
12.5 : 100	0.567	2.463	0.063	0.501
25.0 : 50	-0.803	2.463	0.126	0.711
25.0 : 100	-0.493	2.463	0.048	0.436
50.0 : 100	0.31	2.463	0.019	0.276

*one factor ANOVA where Y=A+BX and X=treatment (ppm)
Y=protein mg/ml

APPENDIX H

TABLE H7 STATISTICAL ANALYSIS

THE EFFECT OF DIETARY AROCLOR 1254 ON THE ACTIVITY OF 7-ETHOXYRESORUFIN IN BUDGIE LIVERS

TREATMENT	N	MEAN	STD. DEV.	STD. ERROR
0.0 ppm	3.0	0.3	0.2	0.1
12.5 ppm	3.0	72.2	35.2	20.3
25.0 ppm	3.0	47.0	5.5	3.2
50.0 ppm	3.0	71.6	16.5	9.5
100.0 ppm	3.0	62.3	32.8	18.9
		ANOVA**		
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F-TEST
BETWEEN GROUPS	4.0	10764.1	2691.0	5.1
WITHIN GROUPS	10.0	5240.0	524.0	P=0.0
TOTAL	14.0	16004.2		
COMPARISON	MEAN DIFF.	FISHER PLSD	SCHEFFE F-TEST	DUNNETT T-TEST
0.0 : 12.5	-71.9	41.65*	3.702*	3.8
0.0 : 25.0	-46.7	41.65*	1.60	2.5
0.0 : 50.0	-71.3	41.65*	3.636*	3.8
0.0 : 100	-62.0	41.65*	2.8	3.3

*significant at 95%; Y=mg resorufin/mg protein/minute
**one factor ANOVA where Y=A+BX and X=treatment (ppm)

APPENDIX H (cont'd)

TABLE H7

COMPARISON	MEAN DIFF.	FISHER PLSD	SCHEFFE F-TEST	DUNNETT T-TEST
12.5 : 25	25.3	41.7	0.5	1.4
12.5 : 50	0.7	41.7	0.0	0.0
12.5 : 100	9.9	41.7	0.1	0.5
25.0 : 50	-24.6	41.7	0.4	1.3
25.0 : 100	-15.3	41.7	0.2	0.8
50.0 : 100	9.3	41.7	0.1	0.5

*significant at 95%; Y=mg resorufin/mg protein/minute
**one factor ANOVA where Y=A+BX and X=treatment (ppm)

APPENDIX I

PREPARATION OF AROCLOR 1254 STANDARDS

1. 47.3 mg of Aroclor 1254 was weighed and dissolved in 100 ml of toluene:ethyl acetate (1:3), T:EA = 473 ppm PCB.
2. 1 ml of 473 ppm PCB was brought to 100 ml with T:EA = 4.73 ppm PCB.
3. 1 ml of 4.73 ppm PCB was brought to 2 ml with T:EA = 2.365 ppm PCB.
4. 1 ml of 2.365 ppm PCB was brought to 10 ml with T:EA = 0.2365 ppm PCB.
5. 1 ml of 2.365 ppm PCB was brought to 2 ml with T:EA = 1.18 ppm PCB.
6. 1 ml of 4.73 ppm PCB was brought to 7 ml with T:EA = 0.676 ppm PCB.
7. 1 ml of 473 ppm PCB was brought to 20 ml with T:EA = 23.65 ppm PCB.
8. 1 ml of 23.65 ppm PCB was brought to 14 ml with T:EA = 1.69 ppm PCB.
9. 45.7 mg of Aroclor 1254 was weighed and dissolved into 100 ml of T:EA = 457 ppm PCB.
10. 1 ml of 457 ppm PCB was brought to 50 ml with T:EA = 9.14 ppm PCB.
11. 1 ml of 9.14 ppm PCB was brought to 10 ml with T:EA = 0.914 ppm PCB.
12. 1 ml of 9.14 ppm PCB was brought to 8 ml with T:EA = 1.143 ppm PCB.
13. 1 ml of 9.14 ppm PCB was brought to 6 ml with T:EA = 1.523 ppm PCB.
14. 1 ml of 9.14 ppm PCB was brought to 4 ml with T:EA = 2.285 ppm PCB.
15. 1 ml of 1.523 ppm PCB was brought to 2 ml with T:EA = 0.762 ppm PCB.
16. 10 mg of Aroclor 1254 was weighed and dissolved into 100 ml of T:EA = 100 ppm PCB.
17. 10 ml of 100 ppm PCB was brought to 20 ml with T:EA = 50 ppm PCB.
18. 10 ml of 100 ppm PCB was brought to 40 ml with T:EA = 25 ppm PCB.

APPENDIX J

PREPARATION OF SPIKED TISSUE

1. 100 ppm PCB = 100 ug/ml PCB. A 10 ml sample of the 100 ppm PCB standard was used to spike 1 gram of control carcass tissue. Calculations are as follows: 10 ml x 100 ug/ml = 1000 ug PCB.

2. 100 ppm PCB = 100 ug/ml PCB. A 5 ml sample of the 100 ppm PCB standard was used to spike 1 gram of control carcass tissue. Calculations are as follows: 5 ml x 100 ug/ml = 500 ug PCB.

3. 100 ppm PCB = 100 ug/ml PCB. A 2.5 ml sample of the 100 ppm PCB standard was used to spike 1 gram of control carcass tissue. Calculations are as follows:
2.5 ml x 100 ug/ml = 250 ug PCB.

APPENDIX K

PREPARATION OF LOWRY REAGENTS

A. STOCK REAGENTS

1. 1% Copper Sulfate ($CuSO_4$) solution: 2.5 g $CuSO_4$ in 250 ml DDH_2O. Store in refrigerator.

2. 2% Sodium or Potassium Tartarate solution: 5 g sodium or potassium in 250 ml DDH_2O. Store on shelf.

3. 10% Sodium Carbonate (Na_2CO_3) in 0.5 M Sodium Hydroxide (NaOH): 100 g Na_2CO_3 in 1 liter of 0.5 M NaOH. The latter was prepared from 20 g NaOH in 1 liter DDH_2O. Store on shelf.

4. Folin & Ciocalteu's Phenol Reagent (2N): purchased through Sigma Chemical Company, #F-9252. Store on shelf.

5. BSA Standard: 500 ug bovine serum albumin/ml DDH_2O. Store on shelf.

B. WORKING REAGENT

1. Copper Solution: ratio of 1:1:20 of stock reagents 1, 2 and 3 above. One ml copper solution per sample.

2. Phenol Solution: ratio of 1:10 of #4 above and DDH_2O, i.e., 10 ml Folin Reagent and 100 ml DDH_2O. Three ml phenol per sample.

APPENDIX L

PREPARATION OF EROD REAGENTS AND CALCULATIONS

1. ASSAY REAGENTS

A. 0.1 M Hepes Buffer (pH 7.8): 23.83 g in 1 liter DDH_2O, adjust pH with 5% NaOH.

B. Generating System: total volume for 100 samples.

1. To approximately 100 ml of Hepes buffer add:
2. 0.0753 g anhydrous $MgSO_4$
3. 0.2 g bovine serum albumin, BSA
4. 0.19 g glucose-6-phosphate
5. 0.364 g NADP

Chemicals 2-5 may be purchased through Sigma Chemical Company. Store the generating system in the freezer for up to a maximum of 3 months.

C. Glucose-6-Phosphate Dehydrogenase (G6PDH): a stock solution of 25 units/ml DDH_2O. G6PDH is purchased through Sigma and stored in the refrigerator for one month.

D. Ethoxyresorufin Solution: A 100 uM solution is prepared.

1. 7-ethoxyresorufin is available in 1 mg quantities from Pierce Chemical Company, MW=242. One mg 7-ethoxyresorufin is dissolved in 41.32 ml methanol. Storage is in an air-tight vial in the freezer. Ethoxyresorufin is very photosensitive, and all solutions should be stored in foil wrapped containers.

2. RESORUFIN STANDARD

A. A stock solution of 1uM resorufin in methanol was prepared by mixing 1 pmol of resorufin to 1 ul of methanol. When running the standard curve, various amounts of resorufin stock solution was added to a clean cuvette; appropriate quantities range from 10 pmol to 250 pmol which equal 10 to 250 ul. Hepes buffer was added to bring the total volume to 2.5 ml. The contents in the cuvette was covered with parafilm and inverted several times. The fluorescent readings were recorded.

APPENDIX L (cont'd)

3. CALCULATIONS

A. Results are expressed as quantity of resorufin formed per quantity of protein in the reaction mixture per unit incubation time multiplied by a volume adjustment factor. Since only 2.5 ml of the 3.75 ml volume is read in the fluorometer, then the total protein produced must be calculated by increasing the total in 2.5 ml to 3.75 ml using a factor of 1.5, i.e. 3.75/2.5=1.5. A sample calculation is (0.95/0.1/20)x1.5=0.713.

TOTAL VOLUME	**FLUOROMETRIC VOLUME**
1.0 ml generating system	2.5 ml in the cuvette
0.2 ml sample mixture	
0.1 ml G6PDH	
0.1 ml ethoxyresorufin	
2.35 ml chilled methanol	

3.75 ml total volume	

3.75/2.5=1.5

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