ABSTRACT

THE EFFECTS OF VARIOUS CHLORINATED HYDROCARBONS ON THE CARDIOVASCULAR PHYSIOLOGY AND HEMATOLOGY OF THE DOMESTIC FOWL

By

Sergio J. Iturri

Polychlorinated hydrocarbons, such as PCB's, DDT and cyclodiene insecticides have called the attention of scientists to the possible hazardous effects that these compounds may have on different animal species, including man himself, due to their ubiquity, resistance to degradation and solubility in hydrocarbon solvents and oils.

This study was carried out to determine the effect of DDT, endrin and various polychlorinated biphenyls on cardiovascular physiology and hematology as well as some toxicological symptoms of the domestic fowl at both lethal and sublethal levels.

Feed intake, mortality, body and organ weights, hydropericardium, heart rate, mean blood pressure, arterial blood pH, packed cell volume, hemoglobin concentration, total erythrocyte concentration, mean corpuscular volume, mean corpuscular hemoglobin concentration, electrocardiogram, electrical axis, cardiac output, stroke volume, peripheral

resistance, Na^+ and K^+ concentration in plasma and pericardial fluid, thyroxine concentration in plasma, as well as, histological evaluation of testes and thyroids were determined in these birds.

In a series of chronic dosage experiments DDT (500-2000 ppm) and endrin (8-20 ppm) were incorporated into the ration of the Single Comb White Leghorn (SCWL) adult female. A significant decrease in hematocrit (HCT) values and hemoglobin concentration were observed in these chickens when DDT (2000 ppm) was incorporated into the diet. In contrast, endrin (16-20 ppm) caused a significant increase in these two parameters. Since total erythrocyte concentration showed the same trend as HCT values and hemoglobin concentration, decreasing with DDT, and increasing with endrin, it seems that these two compounds would be acting, in general, inhibiting or stimulating erythropoiesis. Neither DDT nor endrin produced any change on heart rate, arterial blood pressure or arterial blood pH at any level fed.

In acute dosage experiments, endrin infusion (8 mg/Kg body weight) produced a marked bradycardia and hypertension. These cardiovascular changes were accompanied with convulsions and excessive salivation. It seems that a stimulatory action of endrin on the (parasympathetic and sympathetic) nervous systems occurred. No significant differences in packed cell volume, total erythrocyte concentration and hemoglobin

concentration were observed, before or after endrin infusion in SCWL adult females.

Toxicological symptoms observed in cockerels after chronic oral administration (50-200 ppm) of various PCB's included 1) depressed body weight as a result of decreased feed intake, 2) general edema and hydropericardium, 3) increased liver weight and decreased heart, spleen, and testes weight, 4) depression of the secondary sexual characteristics, and 5) a varying degree of mortality. Also, histological observations showed thyroids of PCB-treated birds to have lymphoid cell infiltration in the connective tissue in an irregular fashion whereas, the rest of the gland presented a fairly normal appearance. Histological examination of testicular tissue revealed an atrophic state of the seminiferous tubules with few undifferentiated germinal cells, no evidence of spermotogenesis, few Leydig cells and the presence of eosinophils in the interstitial tissue.

Of the PCB's tested, PCB's 1242 and 1254 at $\geq$ 100 ppm in the diet significantly reduced heart rate whereas no alteration was found with PCB 1016, 1221 or 1260 at 150 ppm. Mean blood pressure was significantly decreased with PCB 1242 at 100 ppm (pair-fed experiment). Even though this parameter was not significantly different in early studies using PCB 1242 or 1254, it seemed that blood pressure tended to drop when PCB levels increased. No detrimental effect on

blood pressure was observed with PCB's 1016, 1221 or 1260 at any level fed.

PCB's 1242 and 1254 produced abnormal electrocardiograms in cockerels. The voltage of the S waves were lower in leads II and III than those from control groups. Also, some birds fed PCB 1242 showed S-A block, variable amplitude of S wave in an irregular fashion with prominent T or P waves and inverted T waves. Electrical axis, determined by the RS complex, showed no significant change with PCB's 1242 or 1254 at the level fed.

PCB 1242 fed at a rate of 100 ppm did not produce any significant effect on cardiac output, stroke volume and peripheral resistance of SCWL cockerels.

Hemoglobin concentration, HCT and total erythrocyte concentration were found to be significantly decreased by PCB's 1242 and 1254 at $\geq$ 50 ppm. A significant decrease in arterial blood pH was observed with PCB 1254 at 150 ppm. These same parameters were not affected by PCB's 1221, 1260 and 1016 at any level used. It was concluded that the anemia observed was due solely to a decrease in total erythrocyte concentration. The possibility that these changes may be due to a decrease in erythropoiesis was discussed.

Plasma K^+ concentration was significantly higher in cockerels fed PCB 1242 at 100 ppm; whereas Na^+ concentration

was unchanged. On the other hand, both Na^+ and K^+ concentration in pericardial fluid was significantly higher, when they were compared with plasma levels in the PCB-treated birds.

There was a significant relationship between the dietary level of PCB's and the degree of hydropericardium; however, there was no correlation within individual treatments between the magnitude of the bardycardia and the amount of pericardial fluid.

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DEDICATED TO

My wife Gloria, for her endless courage and determination to overcome difficult times.

My family, whose encouragement has been so meaningful in achieving this degree.

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INTRODUCTION

The presence of chemicals in the environment has become a problem of primary concern. Because of the urgent need for food supply, man began using pesticides to improve and protect agricultural production. However, for a majority of pesticides we have only a superficial knowledge with respect to the effects of their long-term use on the ecosystem and the possible consequences on living organisms. On the other hand, all the waste products from a highly technological society are incorporated to the environment, and unquestionably, are becoming part of it. Because no chemical is without biological effect, the concentration accumulated by living organisms and the increasing amount dispersed throughout the world have called the attention of scientists, government officials and the general public to the possible hazardous effects that these compounds may have on different animal species, including man himself. Among the synthetic pollutants which are accumulated by wildlife, the chlorinated hydrocarbon pesticides and the polychlorinated biphenyls appear to be the most abundant. Appropriate attention must be given to other classes of environmental pollutants which might be similarly accumulated by organisms,

similarly spread in the global ecosystem, and which may enhance the effect of those already in existence.

Evidence of the transport and distribution of these chemicals to areas far from the point in which they had been used indicates how serious this problem might become. It has been shown that small, but measurable, traces of DDT* could be found in the bodies of Eskimos in the Artic region where presumably little or no DDT had been used (Durham et al., 1961; Hayes, 1966).

Numerous reports which have appeared in the literature have implicated polychlorinated biphenyls and chlorinated hydrocarbon insecticides as being responsible for producing toxic effects in reproduction and metabolism and pathological changes in different organs and tissues of various animal species and man. However, in comparison, not too many studies have been performed in relation to cardiovascular and hematological changes after exposure to these chemicals.

This study was carried out in an attempt to demonstrate the effect that acute and chronic exposure to PCB's and chlorinated hydrocarbon insecticides may have on some hematological and cardiovascular parameters of the domestic fowl.

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*See Appendix for chemical name.

OBJECTIVES

1. To determine the effects of selected chlorinated hydrocarbons on some cardiovascular and hematological parameters of the domestic fowl after chronic and acute exposure.

2. To establish if the possible changes observed resulted because of the direct action of these chemicals on the cardiovascular system or indirectly through other systems.

3. To evaluate some toxicological aspects of these compounds that may contribute to the overall knowledge of their action on avian species.

REVIEW OF THE LITERATURE

I. GENERAL

A. Polychlorinated biphenyls

Since Jensen (1966) called attention to a "new chemical hazard", intensive research has been done in many areas in order to clarify and understand a family of chemical compounds known as polychlorinated biphenyls (PCB's). These PCB's have become disturbingly widespread throughout the environment, and, because of the similarity with DDT in chemical structure and biological action on organisms, concern has increased in relation to the possible harmful effects of PCB's on different animal species and man. The ubiquity of PCB's resembles that of DDT and it seems that this similarity of distribution is far from being accidental since just recently it has been reported that DDT vapor can be converted to PCB's by irradiation using UV light of the same wavelength present in sunlight in the lower atmosphere (Maugh, 1973).

PCB's were first detected in some species of Swedish wildlife (Widmarck, 1967; Jensen et al., 1969) and subsequently found in birds of Great Britain (Holmes et al., 1967), in fish and birds from the Netherlands (Koeman et al., 1969),

in both terrestrial and marine species of wildlife in the United States (Risebrough et al., 1968), and polar bears of Hudson Bay (Jonkel, 1971).

Polychlorinated biphenyls were first described in the literature in 1881 (Schmidt and Shultz, 1881) but their commercial possibilities and physical characteristics were described by Penning (1930). PCB's are manufactured by Monsanto Company in the United States under the tradename Aroclor, by Prodelee in France as Phenochlor; by Bayer in Germany as Clophen. Other manufacturers are located in Japan (Kanechlor), the Soviet Union, Great Britain, Italy and Czechoslovakia. PCB's available as Aroclors are commonly designated by numbers; the first two digits represent the molecular type: 12=chlorinated biphenyls. The last two digits give the weight per cent of chlorine (Monsanto Technical Bulletins). The commercially available biphenyls range from 21% to 68% chlorine. PCB's are colorless, oily liquids (up to 60% chlorination) with variable viscosity; those with a chlorine content above 60% are solid compounds. They are insoluble in water but soluble in hydrocarbon solvents and oils; therefore, being fat soluble, they are mostly stored in the lipids of animals. Apparently they are more stable than DDT and its metabolites because the ethane molecule between the two phenol rings where most of the transformations of DDT occur is not present in the PCB's (Peakall and Lincer, 1970). They have been widely used in industry as

electrical capacitors, in electrical transformers, hydraulic fluids, heat transfer systems, paint, plasticizers in synthetic resins, pesticide-extenders, inks, and carbonless reproducing paper.

Different isolated incidents where PCB leaked into the environment contaminating different animal species, including man, have been reported in the literature (Harris and Rose, 1971; Veith and Lee, 1971; Kolbye, 1972; Kuratsune et al., 1972) implicating polychlorinated biphenyls as the agents responsible for the toxic effects.

B. Chlorinated hydrocarbon insecticides

DDT, the most widely used pesticide, was synthetized by Zeidler in 1874, but its insecticidal properties were discovered by Paul Müller in 1939 (Metcalf, 1955). Because of special characteristics such as prolonged stability resulting in long-time activity, low toxicity to mammals and wide range of insecticidal action (Metcalf, 1955) the compound became very important as a powerful agent in the control of certain pests. This material was extensively used by the United States Army during World War II for the control of the vectors responsible for typhus and malaria and by farmers as a very potent weapon for the control of agricultural pests.

Other organochlorine pesticides were introduced a few years later. Cyclodiene insecticides which comprise compounds

such as chlordane*, heptachlor*, aldrin*, dieldrin*, isodrin* and endrin* were discovered and developed by the work of Julius Hyman and associates in 1945 (Metcalf, 1955).

For almost 30 years, millions of tons of DDT and other pesticides have been widely used over the world. The consequence was an increasing mortality among wildlife (Bossenmaier, 1959) based on reports of bird mortality following spray programs, threatening animal populations (Rudd and Genelly, 1956). The increasing production of organochlorine pesticides in recent years, have resulted in numerous reports in the literature on pesticide residues indicating that these compounds are scattered throughout the world affecting different animal species and man (Tatton and Ruzicka, 1967; Jensen et al., 1969; Edmundson et al., 1970; Radomski et al., 1971; Zitko and Choi, 1972; Gaskin et al., 1973).

Organochlorine pesticides are essentially insoluble in water, but soluble in various organic solvents, oils and fats. It is their fat solubility and resistance to degradation which are responsible for their prolonged retention and storage in biological material. They accumulate in body fat (Laug et al., 1951; Hayes et al., 1956; Conley, 1960) and fatty tissue of the liver, kidney and brain (Laug, 1948). Acute or chronic exposure to chlorinated hydrocarbon

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*See Appendix for chemical name.

pesticides may be fatal in animals (Gowdey et al., 1952; Blazques et al., 1957; Ecobichon and Saschenbrecker, 1968; Tucker and Haegele, 1971) or man (Negherbon, 1959; Hayes, 1963) whether absorption is by ingestion, by inhalation or dermal (Cameron and Burgess, 1945; Negherbon, 1959; Tucker and Haegele, 1971). Thus, organochlorine pesticides have been implicated as responsible for many biological changes in organisms exposed to them at lethal and sublethal levels.

II. CHLORINATED HYDROCARBONS AND CARDIOVASCULAR SYSTEM

McNamara et al. (1946), reported that daily oral administration of DDT (150 to 300 mg/Kg) to dogs elicited an increase in cardiac output and systolic discharge associated with a decline in arterio-venous oxygen difference. Similar results were shown by Hinshaw et al. (1966), when lethal doses of endrin dissolved in 95% ethanol were injected intravenously in anesthetized dogs. In the same work, a marked and progressive increase in venous return was observed associated with a steady drop in resistance. Also, pulmonary artery pressure increased but pulmonary vascular resistance was unchanged because of a large increase flow through the pulmonary bed.

Emerson et al. (1964), found that lethal amounts of endrin (19 mg/Kg body weight) when infused intravenously into dogs caused hypertension and severe bradycardia. Since bradycardia preceded hypertension, these investigators

concluded that this fact was an indication of the independence of these two phenomena. The fact that bradycardia, after endrin administration, was reversed with atropine, Emerson et al. (1964), suggested that this effect may result from increased vagal activity and/or potentiation of acetylcholine. This decrease in heart rate had been previously reported by Gowdey et al. (1952). They showed that aldrin, when injected intra-arterially into a cat, elicited a marked bradycardia suggesting that aldrin acted as an anticholinesterase when introduced into the circulation. Also intravenous administration of dieldrin in cats caused a marked bradycardia and this increase in cardiac slowing and fall of blood pressure was pronounced when the vagi were intact. These effects were abolished by vagal section (Gowdey et al., 1954). In contrast, Henderson and Woolley (1970) have reported a gradual increase in the heart rate of the adult rat orally intubated with purified p,p'-DDT dissolved in cottonseed oil. Jefferies et al. (1971), found similar results with Bengalese finch. Pulse rate increased directly with the dose rate of p,p'-DDT. However, p,p'-DDT in homing pigeons caused an increase in the pulse rate which then decreased with increasing dose rate. On the other hand, no correlation was found between blood pressure and pulse rate and exposure to p,p'-DDT in human volunteers (Hayes et al., 1971). Also, Rumsey and Bond (1972) reported that there was

no apparent effect on the heart rate of heifers fed aldrin at the rate of 1 mg/Kg body weight. Simpson et al. (1972), found no adverse effects on the blood pressure of turkeys fed DDT supplemented diet. Reins et al. (1966), have shown that the rise in systemic arterial blood pressure in dogs receiving endrin depends primarily on increased cardiac output due to an elevated venous return, the abdominal viscera being the primary source of this increase. In the same study, bradycardia and hypertension were also present confirming the results obtained by Emerson et al. (1964). Emerson and Hinshaw (1965) have shown an increased vascular resistance in the innervated isolated dog's forelimb after endrin infusion. They have pointed out that most of the early increase occurred in the small vessel segment (small artery to small vein), while the arteriole segment contributed the major resistance to blood flow during the later phase. In the same study similar results were obtained after endrin infusion in the denervated forelimb of the dog. They stated that sympathetic innervation of limb vessels apparently was not necessary for the increase in resistance which followed endrin administration. However, experiments where nerve section was performed after endrin indicate that the nerves are responsible for a considerable portion of the total increase in resistance. Reins et al. (1964), found that dogs developed systemic hypertension and increased renal

vascular resistance after intravenous infusion of endrin. These effects were attributable to a sympatho-adrenal action. Acute effects of endrin were predominantly afferent arteriolar vasoconstriction evidenced by decreases in renal blood flow, glomerular filtration rate and urine flow. Adrenalectomy partially offset the marked drop in renal blood flow after endrin, although systemic hypertension and bradycardia were unaffected.

Osorio and Kraemer (1958) reported that dogs with adrenal atrophy produced by technical DDD* showed decreased blood pressure responses to epinephrine and acetylcholine. However, Cueto (1970) has shown that diastolic blood pressure and heart rate response to epinephrine and norepinephrine was similar in both the p,p'-DDD treated and untreated dogs, though a significantly decreased basal blood pressure and heart rate developed in the treated animals during the experimental procedure. Also a significant decrease in the contractile force response to various dosages of epinephrine and norepinephrine was observed in the o,p'-DDD treated dogs compared to the controls. Philips and Gilman (1946) reported that the majority of dogs killed by DDT infusion died of ventricular fibrillation and that some fatally poisoned rabbits, cats and monkeys died by the same mechanism. When DDT was injected intravenously into curarized dogs the

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*See Appendix for chemical name.

myocardium was sensitized so that intravenous injection of epinephrine caused ventricular fibrillation. Curarization paralyzed the animals completely, thus no motor manifestations of the convulsant activity of DDT were evident. This precluded the possibility that myocardial anoxia, which may result from the high oxygen demands accompanying a convulsion in a non-curarized animal, could contribute to the onset of fibrillation (Philips et al., 1946). In the same study, monkeys which received 75 mg of DDT per Kg of body weight showed a marked cardiac arrhythmia, but no fibrillation. Besides fibrillation, premature systoles and changes in the T-wave, frequently involving inversion in all leads, were recorded in dogs. Cardiac arrhytmias have also been observed in acutely poisoned rabbits (Judah, 1949; Deichmann et al., 1950). Danopoules et al. (1953), have recorded abnormal electrocardiograms in human patients severely poisoned with hexachlorocyclohexane. Jefferies et al. (1971), found that pigeons given DDT (3 mg/Kg/day) showed an increase in the amplitude of the S wave. With increasing dose rate there was a decrease in amplitude until at 36 mg/Kg/d, the S wave amplitude showed a 19% decrease below the initial level. Rumsey and Bond (1972) reported that a short-term toxicological effect of aldrin in heifers was indicated by a fluctuation of the T amplitude during the first few months following the initial feeding of aldrin and that

continuous feeding of aldrin increased QRS intervals. On the other hand, no apparent abnormalities in the EKG of the rat were observed during the progression of DDT intoxication (Henderson and Woolley, 1970).

III. CHLORINATED HYDROCARBONS AND HEMATOLOGICAL PARAMETERS

Since chlorinated hydrocarbons have been considered as potential hazards for different animal species and man, the importance of blood in their transport throughout the body has to be taken into account as having a primary role in the intoxication and storage of these compounds. Moss and Hathway (1964) showed that telodrin* and dieldrin in rat and rabbit blood appear in approximately the same concentrations in erythrocytes and plasma. Morgan et al. (1972), concluded that less than 18% of the p,p'-DDT and p,p'-DDE* found in human blood is carried in the erythrocytes while dieldrin is distributed between red blood cells and plasma roughly in proportion to volume. Also, the plasma albumin and, secondarily, the smaller globulins are the principal plasma protein constituents associated with blood-borne p,p'-DDT and p,p'-DDE. Dale et al. (1965), suggested that the binding of pesticides to serum protein was the cause of incomplete recovery from human serum by hexane extraction. Also binding of dieldrin and telodrin to serum proteins has been

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*See Appendix for chemical name.

demonstrated by Moss and Hathway (1964). Schoor (1973) showed that p,p'-DDE is bound to human serum proteins in large amounts. Moreover, Mick et al. (1971), reported that dieldrin residues in α- and β-lipoprotein fractions of human blood increased at the same rate when plasma dieldrin increased; however, the β-lipoprotein fraction contained more dieldrin than the α-lipoprotein fraction. Endrin, after acute poisoning, was regularly detected in human plasma (Curley et al., 1970).

Exposure to chronic doses of DDT has been reported to decrease hemoglobin concentration in rabbits (Cameron and Burgess, 1945) and dogs (McNamara et al., 1946) without significant reduction in the total number of erythrocytes while Burlington and Lindeman (1950) showed that chronic DDT injections in cockerels did not change hemoglobin concentration but number of red blood cells was reduced by an average of 17.8 percent. Wright et al. (1946), reported agranulocytosis in humans occurring after exposure to DDT. Friberg and Martensson (1953) have described the occurrence of pancytopenia and marrow hypoplasia in humans following exposure to a spray containing DDT and lindane.* The hematologic changes began with granulocytopenia in the first week following exposure with thrombocytopenia and anemia appearing a week later. Anemia has been reported in humans exposed for

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*See Appendix for chemical name.

a prolonged period of time to DDT, lindane and chlordane (Moore, 1955). Also aplastic anemia has been observed in human patients severely poisoned with DDT and lindane (Sanchez-Medal et al., 1963; Loge, 1965). Mastromatteo (1964) indicated that human subjects developed blood dyscrasias after exposure to chlorinated hydrocarbons. Anemia and increased sedimentation rate were observed in human patients after serious poisoning by hexachlorocyclohexane (Danopoules et al., 1953). Draize et al. (1944), found that rats occasionally showed a fall in hemoglobin after exposure to DDT. In contrast, Neal et al. (1946), and Hayes et al. (1971), reported no changes in the hemoglobin content and red blood cell count on man after a single oral dose (770 mg) or long-term oral doses of DDT. However, Velbinger (1947) found that oral doses of 500 to 1,500 mg in man decreased hemoglobin concentration and total erythrocyte count. Deichmann et al. (1950), indicated that changes in the hemoglobin or in the formed elements of the blood are not always present even in severe poisoning.

A decrease in hemoglobin concentration accompanied with a decrease in packed cell volume of chickens has been reported by Flick et al. (1965), as early as one week of age after feeding PCB (42% chlorinated) at 200 and 400 ppm in the diet. Rehfeld et al. (1972a,b), using one-day old cockerels, observed a drop in hemoglobin concentration and

hematocrit when feeding PCB 1248 at levels as low as 20 and 30 ppm for 4½ weeks. These changes were reversible when the PCB was withdrawn after 2½ weeks. Similar results have been shown by Abrahamson and Allen (1973) in monkeys intubated daily with 35 mg/Kg of Aroclor 1248. Emerson et al. (1964), found an increase in the packed cell volume of dogs acutely poisoned with endrin. This increase in hematocrit appears to result in part from addition of cell-rich blood from the spleen (Emerson, 1965). Continuous dietary administration of DDT resulted in a decrease in the packed erythrocyte volume and the total erythrocyte count of the blood of mature male Japanese quail (Ernst and Ringer, 1968). However, Cueto (1970) and Rumsey and Bond (1972) reported no changes in the packed cell volume of dogs after oral administration of o,p'-DDE or of beef heifers after being chronically fed with aldrin.

Draize et al. (1944), reported that animals receiving dermal applications of DDT showed a moderate leucocytosis with a definite increase in the percentage of heterophiles. Cameron and Burgess (1945) found that after large dermal applications of DDT, a pronounced leucocytosis appeared in rabbits on the second or third day after exposure, however, Davignon et al. (1965), reported a greater incidence of leukopenia in apple growers exposed to pesticides than in control populations. Velbinger (1947) had reported that a man after being exposed to high doses of DDT did not show

either an immediate or a delayed leukopenia. Rather, there was in most instances, a slight leukocytosis. There was no evidence of significant changes in white blood cell count after long-term, high, oral doses of DDT in man (Hayes et al., 1971). Emerson et al. (1964), have found leukocytosis in dogs acutely poisoned with endrin. Kunev (1965) reported an increased phagocytic action in the blood (leukocytosis) of rats when relatively small amounts of DDT were injected intragastrically.

IV. CHLORINATED HYDROCARBONS AS AFFECTING BODY AND ORGAN WEIGHTS

Numerous reports show that chlorinated hydrocarbons are capable of producing changes affecting body weight and various organs in different animal species and man.

A. Polychlorinated Biphenyls

Bennet et al. (1938), reported that rats which had been given six daily doses (300 mg) of a chlorinated hydrocarbon (65% chlorinated) showed an increase in liver weight and hepatic cell swelling. Treon et al. (1956), found that rats after vapor exposure to a chlorinated biphenyl (54% chlorinated) showed liver cell injuries and increased liver weight. Platonow and Funnell (1971) observed an increase in liver weight of cockerels when PCB 1254 was incorporated in the ration at 150-250 ppm for six to thirteen weeks. Also, Grant et al. (1971), have shown that PCB 1254 produced an increased

liver weight in the rat. They suggested that the liver is the main site of Aroclor 1254 metabolism since rats with carbon tetrachloride damaged livers were unable to metabolize this mixture of chlorinated biphenyls as rapidly as rats with normal livers. Lincer and Peakall (1973) observed that ring doves after a daily dietary dose of 10 ppm of PCB 1254 tended to lose relatively more weight than the controls. An increase in liver weight was observed in chickens fed PCB 1242 at a rate of 100 to 1000 ppm in the diet for three-four weeks (McCune et al., 1962; Flick et al., 1965) and in pigeons fed 500 ppm during a similar period of time (Bailey and Bunyan, 1972). Also, Bitman et al. (1972), and Cecil et al. (1973), showed that PCB 1242 produced an increase in liver weight in male and female rats and male Japanese quail. Liver damage had been reported previously in different mammals over a short period of time after dermal exposure (35 mg daily) with PCB 1242 (Miller, 1944). Aroclor 1248 fed to ten-day-old chickens depressed growth rates at dietary levels of 50, 100 and 150 parts per million (Rehfeld et al., 1971). These birds showed increased liver weight as a percentage of body weight, primarily as a reflection of lower body weights. Abrahamson and Allen (1973) observed only a slight increase in weight of liver and other organs when expressed in relation to body weight in infant monkeys intubated daily with 35 mg/Kg of Aroclor 1248. Vos and

Koeman (1970) reported that when chickens were kept on rations containing 400 ppm of Aroclor 1260 for 60 days their body weights were significantly reduced, but their liver weights and relative liver weights were significantly increased when compared with control values. Also, Vos and Beems (1971) observed a significant decrease in body weight and increase in relative liver weight in rabbits after dermal exposure to PCB 1260. Dahlgren et al. (1972), found that PCB's increased weights of liver in pheasants given 10 and 20 mg doses but no effect was observed with higher doses. In contrast, Prestt et al. (1970), using the Bengalese finch, reported no changes in liver weight after various doses of PCB 1254.

Organs other than the liver also have been affected by polychlorinated biphenyls. Dahlgren et al. (1972), showed that PCB's decreased weights of heart and spleen at all treatment levels, but increased kidney weight at low doses, without any effect at higher doses. Splenic atrophy has been reported by Flick et al. (1965), and Vos and Koeman (1970) in chickens fed various PCB's (42 and 60% chlorinated, respectively). Splenic atrophy was characterized by almost complete absence of lymphatic nodules and an increase in the relative abundance of red pulp. Also, Grant et al. (1971), reported decreased spleen size over a period of time in rats given PCB 1254. Prestt et al. (1970), using the Bengalese

finch, observed that kidneys were larger in birds that died after dietary ingestion of PCB 1254 at a rate of 400 ppm for 56 days than in controls. Flick et al. (1965), observed both enlarged adrenals and kidneys in chickens after PCB treatment (Aroclor 1242) for three weeks at a rate of 200-400 ppm. Dahlgren et al. (1972), reported an increase in kidney size in pheasants given ten or twenty mg daily of PCB 1254 while Platonow and Funnell (1971) indicated that testes and comb size decreased in chickens when the same PCB was incorporated into the rations at a rate of 150 to 250 ppm. Also, an increase in weight and size of gull thyroid was reported by Jefferies and Parslow (1972) after the birds were dosed daily with 50-400 mg/kg of Aroclor 1254 dissolved in cod liver oil.

B. Chlorinated Insecticides

Treatment or accidental exposure with chlorinated insecticides like DDT-type compounds and those from the cyclodiene group has been shown to bring about gross and microscopic changes in various organs in mammals, birds and man. Studies with rats in which DDT dissolved in corn oil was administered by stomach intubation showed an increase in liver weight and also showed that intoxication with DDT led to an increase in the ether-soluble fraction of the liver (Sarett and Jandorf, 1947). Similar increase in liver weight of rats after DDT ingestion has been reported previously

(Laug and Fitzhugh, 1946). Fitzhugh and Nelson (1947) found a significant increase in liver weights of rats which had been fed a diet containing 400 ppm DDT, however body weight was significantly decreased. In the same study, kidney weights increased significantly at 600 ppm DDT. In contrast, Durham et al. (1963), reported no hepatic changes in monkeys fed for seven years with DDT at levels of 200 ppm or less. Hart and Fouts (1963) were the first to demonstrate that DDT stimulates hexobarbital metabolism in the rat. In another report, Hart and Fouts (1965) showed that doses of DDT that stimulated drug metabolism also increased liver weight. On the other hand, Klion (1966) has reported a biphasic response of the liver after dieldrin administration. In small doses this compound induces drug-metabolizing enzymes while larger amounts result in depression of induction and mitochondrial injury. Hoffman et al. (1970), found that liver weight increased in proportion to the dose when rats were maintained on diets containing 128-512 ppm DDT. Levels less than 128 ppm had no effect and concentrations greater than 512 ppm produced a submaximal increase in liver weight. In the same study, these researchers suggested that stimulation of drug-metabolizing enzymes and the increase in liver weight might be a manifestation of physiological adaption. DDT increased liver weight and lipids of male and female rats or male Japanese quail (Cecil et al., 1973).

Boyd and Stefec (1969) have reported that albino rats given toxic levels of endrin showed changes in the wet weight of organs at death. At autopsy there were degenerative changes in the kidneys and liver, loss of weight and dehydration of many organs. Lawrence et al. (1968), found that single oral dose of endrin (25 mg/kg body weight) in rats produced a significant increase in weight of the liver, kidney, brain and heart but not the spleen. Also, Treon et al. (1955), found that dogs fed endrin at eight ppm for almost six months had enlarged liver, kidneys and brain and rats given 5-25 ppm for two years showed that liver weight expressed as percentage of body weight was significantly increased when compared with those from the control group. Harr et al. (1970), described cytoplasmic changes of the liver in female rats after dieldrin exposure and the hepatic lesions observed were associated with increased hepatic weight. Reins et al. (1964), reported congestion and swelling of the liver, lungs and spleen following acute or chronic poisoning in dogs. Nelson and Woodard (1949) observed adrenal cortical atrophy and fatty degeneration of the liver of dogs fed 50 to 200 mg of DDD per kg body weight per day. Fitzhugh and Nelson (1947) claimed that a slight generalized increase in the size of the adrenal occurred in rats fed for two years on DDT. Bengalese finches developed apparent hyperthyroidism following ingestion of p,p'-DDT for six weeks (Jefferies,

1969) and sublethal quantities of the same drug increased thyroid weight and reduced colloid content of follicles in homing pigeons (Jefferies and French, 1969). Richert and Prahlad (1972) reported a significant increase in the absolute weight of thyroid glands in Japanese quail after being fed 150 ppm DDE. Thyroid weights were increased in rats fed o,p'- and p,p'-DDD and this effect was interpreted as evidence of a compensatory hypothyroidism (Fregly et al., 1968). Cockerels receiving 5 to 30 mg daily subcutaneous injections of DDT from 8 to 89 days of age showed a reduction in the size and development of the testes (Burlington and Lindeman, 1950).

V. CHLORINATED HYDROCARBONS AND REPRODUCTION

Reproduction seems to be one of the most vulnerable functions in chlorinated hydrocarbon poisoning. This is true in mammals as well as in other living organisms, including birds, which are more sensitive to these chemicals than are mammals.

A. Polychlorinated Biphenyls

Gilbert (1969) and Aulerich et al. (1971) have reported reproductive failure in mink that were fed fish contaminated with PCB's. Ringer et al. (1972) confirmed the previous reports after feeding 10 ppm of various PCB's, or PCB's in combination with DDT and dieldrin, to mink. Dahlgren and

Linder (1971) have shown a decrease in egg production, and a higher percentage of chicks that pipped the shell but did not hatch, in pheasants that received 50 mg of Aroclor 1254 for 17 weeks. In the same study, chicks that hatched weighed less and survived more poorly than controls. Egg shell thickness was not altered. McLaughlin et al. (1963) injected both 10 and 25 mg Aroclor 1242 into chicken eggs and found only 0-5% hatchability, growth retardation and beak deformities in embryos. Peakall (1971), after feeding ring doves 10 ppm of Aroclor 1254 for six months, did not find any changes in eggshell weights compared with those laid by control birds. Injection of birds intraperitoneally with a dose equivalent to 160 mg/kg of PCB 1254 one to four days before egg-laying confirmed the lack of effect of PCB's on shell weight (Peakall, 1971). Based on their studies on pelicans and cormorants, Anderson and co-workers (1969) have concluded that DDE has a greater effect on shell thickness than does PCB's. Heath et al. (1970) reported that mallards were fed 25 ppm of Aroclor 1254 during two breeding seasons without any effect on egg production, eggshell thickness or number of cracked eggs. Scott et al. (1971), who fed 0 to 20 ppm Aroclor 1248 for eight weeks to chickens in full egg production, found no reduction in this parameter on the lowest levels of PCB; however, some reduction in egg production was associated with the higher levels. In Bobwhite quail fed

diets containing 50 ppm of PCB's or 30 ppm of DDE or a combination of both PCB and DDE for 11 weeks, egg production as well as hatchability turned out to be as good as controls (Heath et al., 1970). Dahlgren et al. (1972) found no changes in eggshell thickness in pheasants that received a capsule dose of 50-100 mg of PCB 1254 or combination of PCB and dieldrin for five weeks. In the same study, egg production in the treated birds was not different from that of the control group at lower doses but significantly decreased at higher doses. Platonow and Funnell (1971) have reported that feeding PCB 1254 at 250 ppm level for 13 weeks to White Leghorn cockerels produced a noticeable decrease in testes weight with consequent decrease in comb size. Rehfeld et al. (1971) also found a decrease in the development of comb and wattles in one-day-old cockerels fed diets containing 40 and 50 ppm of PCB 1248 for four and one-half to five weeks. Platonow et al. (1972) showed that boars given high doses of PCB 1254 excreted lower levels of estrogen and dehydroepiandrosterone in the urine. Bitman and Cecil (1970) have described the estrogenic activity of various PCB mixtures, evaluated by the glycogen response of the immature rat uterus, while Nowicki and Norman (1972) reported that pretreatment of chickens with PCB's 1254 or 1260 enhanced the in vitro hepatic metabolism of natural steroid hormones.

B. Chlorinated Insecticides

Fitzhugh (1948) reported that rats fed diets containing 50, 100, and 600 ppm of DDT showed a progressive decline in the percentage of young successfully weaned, as compared with rats fed diets with DDT at 0 or 10 ppm level. Ball et al. (1953) found that giving rats aldrin in the diet at a level of 120 ppm caused their estrus cycle to be disturbed. Intraperitoneal injection of doses of 50 mg/kg purified p,p'-DDT, purified methoxychlor*, technical DDT or purified o,p'-DDD increased uterine wet weight of immature female rats (Welch et al., 1969). Also, Duby et al. (1971) have shown that injection of 1-4 mg of o,p'-DDT increased uterine weights in intact immature rats and in intact and castrate immature mink while the p,p'-isomer had only slight activity. The effect of the technical DDT was dependent upon the level of o,p'-isomer present in it. In the same study, chronic ingestion of these compounds by rats at levels of 1 to 15 ppm did not cause any effect on fertility or fecundity in two successive generations. Wrenn et al. (1970) reported that daily doses of o,p'-DDT at 10 and 50 µg given to 18-day-old female rats exerted a clear estrogenic action in causing early vaginal opening; however, long-term, low level (1 and 2.5 ppm) doses of o,p'-DDT caused no detrimental effect on uterine weight and did not interfere with normal reproduction in young rats. Wrenn et al. (1971) and Ottoboni (1969) found

*See Appendix for chemical name.

no adverse effect on fertility, fecundity or viability of young rats fed 40 ppm o,p'-DDT or 20-200 ppm technical grade DDT. In contrast, Bernard and Gaertner (1964) reported a decrease in reproductive success of mice when they were fed DDT. Ambrose et al. (1953) and Welch et al. (1971) found that chlordane decreases fertility in female rats and female mice.

Chlorinated insecticides have been reported to stimulate the metabolism of sex steroids. Chlordane administration for several days decreases the action of estrogens on the uterus of mice and rats and reduces the growth-promoting effect on the seminal vesicles in the rat (Levin et al., 1969). In addition, the same drug stimulates the activity of liver microsomal enzymes that metabolize testosterone in vitro (Welch et al., 1967). Peakall (1967) has shown that the hydroxylation of progesterone and testosterone by the pigeon liver was increased after feeding DDT and dieldrin. When ring doves were fed 10 ppm DDT, estrogen metabolism by the liver was increased, the concentration of estradiol-17β in plasma was decreased and ovulation time was delayed (Peakall, 1970a). Singhal et al. (1970) demonstrated that administration of o,p'-DDT (10 mg/100 g) to ovariectomized rats resulted in marked increases in uterine weight and in the activities of several uterine carbohydrate metabolizing enzymes; whereas, Fahim et al. (1970) reported that intraperitoneal administration of DDT (15 mg/kg) in sexually mature virgin rats altered

the *in vivo* function of estrogen by decreasing uterine weight. Bitman *et al.* (1968) found increased oviduct weight in Japanese quail after treatment with o,p'-DDT.

Chicken males injected with increasing dosages of DDT, 15 mg/kg at eight days of age to 300 mg/kg at 89 days of age, had reduced comb, wattle and testes weight when they were compared with those from control birds of the same age (Burlington and Lindeman, 1950). In contrast, Simpson *et al.* (1972) after long term feeding of 264 ppm of o,p'-DDT and p,p'-DDT to six week-old Broad-Breasted White Turkeys reported that testes and oviducts were of similar size in birds from all treatment groups, suggesting a lack of estrogenic response after feeding DDT to turkeys. No significant estrogenic effects were found in chickens fed methoxychlor at levels as high as 10 ppm (Foster, 1973). Lillie *et al.* (1972) reported that feeding of 5 and 50 ppm levels of p,p'-DDT and o,p'-DDT to White Leghorn pullets significantly reduced hatchability and that p,p'-DDT, o,p'-DDT and p,p'-DDE at 300 ppm level reduced egg production but did not affect fertility nor hatchability. Weihe (1967) reported that 1000 ppm of DDT were necessary to decrease hatchability of chickens. However, levels as low as 10 ppm of DDE did affect fertility and hatchability of penned Mallard ducks (Heath *et al.*, 1969), suggesting a possible specie difference in response to pesticide intake. DeWitt (1956) found that

egg production, fertility and hatchability were relatively unaffected in Bobwhite quail after DDT feeding. Genelly and Rudd (1956) showed that 25 or 50 ppm dieldrin significantly dropped egg production of pheasants, while egg fertility of birds receiving 50 ppm was lower than that of controls. DDT in the diet of pheasant breeders at 500 ppm level did not reduce egg production, fertility or hatchability, but chicks hatched had significantly higher mortality than did controls, to 46 days of age; however, female Japanese quail fed 500 and 700 ppm DDT showed a drop in egg production and hatchability, but at lower doses these parameters were unaffected (Cross et al., 1962). Ring doves fed a diet containing 40 ppm of p,p'-DDE produced 13.5% fewer egg/clutch than did controls and experienced twice as great mortality of young whereas hatchability was not significantly affected (Haegele and Hudson, 1973). Wilson et al. (1973) just recently reported that Bobwhite quail hens dosed by gelatin capsule twice weekly with 10 or 20 mg DDT did not show any change in egg production; however, the 20 mg dosage treatment resulted in decreased fertility and hatchability.

Eggshell thickness and its relation to chlorinated hydrocarbons has been the object of many studies since Ratcliffe (1967) suggested that the introduction of DDT into general use (about 1945) coincided closely with onset of

eggshell changes. Subsequently, Hickey and Anderson (1968) reported that dramatic declines of three raptorial species of birds in the United States were accompanied by decreases in eggshell thickness that began in 1947. Since then, many investigators have tried to establish the relationship between the changes in eggshell and the presence of these organochlorine compounds observed in the field. Few reviews on the subject have appeared in the literature (Ratcliffe, 1970; Peakall, 1970b); Risebrough et al., 1970). Just recently, Cooke (1973) has reviewed in detail the effect of environmental pollutants on thickness of shells of avian eggs. Related laboratory investigations have helped to clarify the effects of these compounds in the production of abnormal egg shells. Heath et al. (1969) observed that DDE in concentrations of 10 and 40 ppm in the feed of penned mallards induced a significant egg shell thinning and cracking and a marked increase in embyro mortality. Bitman et al. (1969) reported that the feeding of 100 ppm o,p'- and p,p'-DDT to Japanese quail resulted in the production of eggs with thinner shells and lower Ca content than usual, although the pesticide intake of these quails was far in excess of that ingested by seed-eating birds in the field. While most studies have shown that chronic treatments with moderate to high levels of DDT and DDE result in only about 10% thinning, Tucker and Haegele (1970) established that percentage

eggshell thinning may be accomplished by different method of DDT administration. After giving single oral doses of 1000 mg/kg technical DDT by gelatin capsule to mallard hens, they found that 25% thinning in the shells of mallard eggs can be produced by this way. Sauter and Steele (1972) showed that egg shell thickness was significantly reduced in pullets fed DDT and lindane at rates of 0.1, 1 or 10 ppm, while Cecil et al. (1972), concluded from studies with White Leghorn pullets that egg shell thickness and egg shell calcium were unaffected after dietary treatment of 5, 25 and 50 ppm of p,p'-DDT, o,p'-DDT or p,p'-DDE for 28 weeks. Smith et al. (1970), reported that 10 ppm technical DDT reduced egg shell thickness in eggs of laying hens. In contrast, Davison and Sell (1972) found that 100 or 200 ppm p,p'-DDT fed to chickens for a period of 12 weeks had no effect on egg weight, shell thickness or shell calcium. Similar results were obtained when dieldrin was fed to chickens at levels up to 20 ppm (Davison and Sell, 1972). A significant reduction in eggshell quality was recorded in prairie falcons fed dieldrin contaminated starlings (Enderson and Berger, 1970). In addition, sparrow hawks fed a combination of dieldrin and DDT produced eggs with significantly thinner shells (Porter and Wiemeyer, 1969). Peakall (1970), however, reported that dieldrin 20 ppm injected prior to egg laying in the ring dove produced no significant thinning of the

eggshell. Longcore et al. (1971), found that dietary dosages of 10 and 30 ppm of DDE caused significant shell thinning and shell cracking in captive black ducks. Significant decreases in barium and strontium, a decrease in shell calcium, and a significant increase in the percentage of magnesium were found in black duck eggshell composition after the birds had received diets containing 10 and 30 ppm DDE (Longcore et al., 1971). Haegele and Hudson (1973) reported that ring doves fed diets containing 40 ppm of p,p'-DDE for 126 days showed 10% thinner eggshells than untreated birds.

VI. CHLORINATED HYDROCARBONS AND THEIR TOXICITY

The usual way of expressing acute toxicity of a compound is estimating the dose of the compound that would be lethal to 50% of the population tested (LD_{50}); however, this method of evaluation, even though effective as a mortality measurement, does not give any indication of the subtle, yet damaging effects that this substance may have at sublethal levels.

Some of the changes produced by various chlorinated hydrocarbons have already been reviewed in Sections II, III, IV and V, therefore, only specific toxicological aspects of these compounds will be mentioned below.

A. Polychlorinated Biphenyls

Since no definite work has been done to establish LD_{50} values for various PCB's, their toxicology is not as well

known as that of the chlorinated hydrocarbon pesticides. Tucker and Crabtree (1970) using a single dose of PCB 1254 (100 mg/kg by stomach intubation), found that it was fatal to two out of three rats while Aroclor 1268 killed one rat out of three at 500-4000 mg/kg. In contrast, Smyth (1931) reported that 4 g/kg of a chlorinated benzene derivative (degree of chlorination not stated) did not show any toxic effect in rabbits and guinea pigs. Also, Aroclor 1242, 1254 or 1268 at a dose level of 2000 mg/kg was not fatal to mallard ducks (Tucker and Crabtree, 1970). The toxicity of Aroclor 1254 to Bengalese finches has been examined by Prestt et al. (1970). They found that the concentration of PCB's in the liver from the birds that died was 70-697 ppm compared to 3-634 ppm in those that survived. These authors concluded that Aroclor 1254 has only 1/13 the toxicity of DDT; however, the comparison is difficult because of the different shape of the mortality curves for these two compounds. Vos and Koeman (1970), using PCB's (60% chlorinated) supplied by different manufacturers, reported that 400 ppm fed to chickens produced different results in toxicity. They found that the French PCB produced 100% mortality in 12-58 days while complete mortality occurred in 13-29 days with the German product. The mortality for Aroclor (American compound) was only 3/20 for a 60-day period. On the other hand, McCune et al. (1962), observed no mortality after feeding chickens with PCB 1242 at a level of 100 or 200 ppm for

a 4-week period. However, mortality was 50% and 90% when the same compound was fed at 400 and 800 ppm, respectively, in the same period of time. Flick et al. (1965), found that four chickens out of 24 died when they used the same PCB at 400 ppm in the diet in a 3-week period. Rehfeld et al. (1971), reported severe mortality (80%) in chickens fed PCB 1248 at 50 ppm for $4\frac{1}{2}$ to 5 weeks. Total mortality with Japanese quail was observed between 6 and 55 days after feeding a diet of 2000 ppm PCB's (60% chlorinated) (Koeman et al., 1969). Heath et al. (1970), tested six PCB mixtures, containing 32 to 62 percent chlorine, with pheasants, mallard ducks, Bobwhite quail and Japanese quail. They showed that toxicity increased with the percentage of chlorine. Special tests with Japanese quail showed that the toxic effects of DDE and Aroclor 1254 were additive but not synergistic. Dutsman et al. (1971), reported that Aroclor 1254 was approximately as toxic as DDE to four species of blackbirds. Signs of poisoning were sluggishness, slight tremoring and liver showing hemorrhagic spots. A summary of the current knowledge of PCB's and their toxicity has been reported by Peakall and Lincer (1970).

In addition to lethal effects as a result of PCB exposure, sublethal effects are also important. Some of these changes have been mentioned in previous sections. General edema and hydropericardium was observed in chickens fed diets

containing > 20 ppm of PCB 1248 (Rehfeld et al., 1971). McCune et al. (1962), and Flick et al. (1965), reported edema and hydropericardium in chickens fed 400 ppm PCB 1242 and in Japanese quail at 1000 ppm of PCB (60% chlorinated). Also, Prestt et al. (1970), observed that some Bengalese finches showed hydropericardium after receiving Aroclor 1254 in their diets.

B. Chlorinated hydrocarbon insecticides

A number of studies have been carried out to establish LD_{50} values and toxicological effects in relation to chlorinated hydrocarbon insecticides. There are already excellent summaries for LD_{50} values of cyclodiene insecticides (Soto and Deichmann, 1967) and various pesticides (Tucker and Crabtree, 1970). Also, a good review describing the basic pharmacology and toxicology of DDT has been written by Hayes (1959). A correlation has been reported between dieldrin concentrations in the blood and clinical signs of intoxication in both human beings and dogs (Brown et al., 1964). In contrast, the residue levels of DDT and its principal metabolites, DDD and DDE, in brain tissue have been shown to be the best criteria for determining poisoning in rats (Dale et al., 1963), sparrows (Bernard, 1963), and cowbirds (Stickel et al., 1966). A positive correlation was observed between plasma levels of DDT and those found in the brain in rabbits after single intravenous dose at levels of

150, 100, 50, 25 and 12.5 mg/kg (Black and Ecobichon, 1971). These investigators stated that the severity of the clinical signs of DDT poisoning was related with the levels detected in the brain. Dale et al. (1962), indicated that DDT in the blood corresponds more closely to DDT in the brain than in the fat. Using data from various studies in birds, Stickel et al. (1966), proposed 30 ppm of DDD + DDT in the brain as indicative of death or serious danger. Hill et al. (1971), after feeding Bobwhite quail 25 to 800 ppm technical DDT ad-libitum for five days, correlated DDT, DDD and DDE in the brains with dietary concentrations. They suggested that 20 ppm in brain tissue would be critical, since they observed signs of intoxication associated with less than 10 ppm.

Susceptibility to chlorinated hydrocarbon toxicity has been shown to be inversely related to nutritional status. Stickel et al. (1965), showed that woodcocks in good condition, as reflected by body weight, were less susceptible to heptachlor poisoning than were birds in poor condition. Boyd and Krijnen (1969) demonstrated that DDT toxicity was augmented in protein-deficient albino rats (0-3% casein) when compared with animals fed normal amounts of dietary protein (27%). Dale et al. (1962), reported that when rats which had been fed sublethal levels of DDT were subjected to partial starvation, DDT was mobilized from the fat depots into the blood stream and into the brain, resulting in toxic signs.

A similar experiment carried out with cockerels produced similar toxic signs and death (Ecobichon and Saschenbrecker, 1969).

Black and Ecobichon (1971) reported that after a single intravenous dose of emulsified DDT (12.5-150 mg/kg) to female rabbits, tremors and convulsions were observed in all animals receiving more than 12.5 mg/kg. Clonic convulsions have been observed in rats after exposure to 20 and 40 ppm dieldrin (Harr et al., 1970) and in dogs acutely poisoned after intravenous infusion of endrin (Emerson et al., 1964). Garretson and Curley (1969) observed convulsions, heavy salivation, seizures and cyanosis after dieldrin poisoning in humans. Also, salivation has been reported by Emerson et al. (1964), in dogs after endrin poisoning. In contrast, Hayes et al. (1971), found that human volunteers who ingested technical or recrystallized p,p'-DDT at rates of 3.5 and 35 mg per man per day for 12.5 months did not show any definite clinical or laboratory evidence of injury by DDT. They concluded that ". . . these factors indicate a high degree of safety of DDT for the general population."

MATERIALS AND METHODS

I. GENERAL

Three chlorinated hydrocarbons were selected for this study because of their chemical resemblance from a structural point of view. All three show the presence of the benzene ring as heavily chlorinated. (1) DDT (Dichlorodiphenyltrichloroethane) was chosen because it is a well-known insecticide and has been widely used. Its biological effects on different animal species, including man, have been fairly well established. (2) PCB's (Polychlorinated biphenyls) comprise a complex series of chemicals and they were selected because they are capable of producing many biological changes after ingestion by different animal species when they become accidental contaminants. From the standpoint of poultry, PCB's are particularly important because they may produce, among others, symptoms similar to those of "chick edema" disease. (3) Endrin was selected because it is the most toxic member of a group of chemicals with powerful insecticide properties.

II. DIETARY TREATMENT

Diets containing different levels of PCB's* were prepared by adding the respective polychlorinated biphenyl to a chick starter ration (Table 1). The PCB's were dissolved in acetone and mixed into a premix to facilitate an even distribution in the final mixture. The acetone was evaporated and the premix blended into the basal ration. An equal amount of acetone treated premix was mixed with the basal ration for the control groups.

DDT (Tech. 77.2%; City Chemical Corporation, N.Y.), and endrin (Tech. 95%; City Chemical Corporation, N.Y.) were ground and converted into a fine powder by means of a mortar and pestle, weighed and mixed with a known amount of powder from a laying ration (Table 2) which had been screened (U.S. Standard sieve series No. 30; opening 595μ) three times. This premix was combined and mixed thoroughly with the basal ration using a rotating drum mixer to give the final concentration. The same procedure was used for the control groups except that pesticides were not added. Feed and water were available ad libitum except as noted in special cases. Weekly feed consumption was measured on treatment basis. All premix rations were stored in 3½-gallon tin cans (Ellisco Inc., Philadelphia, Pa.) and kept under refrigeration (5°C).

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*Aroclor 1016, 1221, 1242, 1254 or 1260; Monsanto Chemical Co., St. Louis, Mo.

Table 1. Chick starter ration.

Ingredients	Lbs per ton
Yellow corn, fine ground	1060
Ground oats	100
Wheat middlings	100
Alfalfa meal, dehydrated, 17% protein	80
Meat and bone scraps, 50% protein	50
Solvent process soybean meal, 45% protein	500
Fish meal, Vitaproil, 55% protein	40
Dried whey	40
Ground limestone	10
Dicalcium phosphate (24% Ca - 18.5% P)	10
Salt, iodized	6
Vitamin trace mineral premix	5
Coccidiostat	+
	2001 +

Table 2. Laying ration.

Ingredients	Lbs. per ton
Corn, ground yellow	690
Oats	400
Wheat bran	300
Wheat middlings	200
Alfalfa leaf meal, 17% protein	60
Soubean meal, 44% protein	50
Meat scraps	60
Dried skim milk	20
Fish meal	50
Oyster shell flour	100
Steamed bone meal	30
Salt	12
Cod liver oil (400D, 2,000A)	8
	2,000

In order to eliminate feed wastage and have a more accurate estimation of feed and pesticide intake, feeders were half-filled every day.

III. PAIR-FED EXPERIMENT

Three groups of cockerels were housed separately in starting batteries to study the possibility that feed consumption might alter some of the parameters being analyzed. The experimental group was given a known amount of feed where PCB had been incorporated and treated as described previously. Feed consumption from this group was measured on alternating days and an equal amount of the basal ration without PCB was given to the pair-fed group. In this manner the 2-day lag afforded the pair-fed group the same amount of feed consumed by the experimental group. This procedure was followed until the experiment was terminated. A third group was fed the basal ration ad libitum.

IV. HOUSING AND EQUIPMENT

Either one-day-old commercial (DeKalb strain) or pure strain (MSU Research Farm) Single Comb White Leghorn (SCWL) cockerels were used for PCB studies. They were reared in starting batteries adjusted to 35°C, with raised wire floors. The birds were weighed individually, wing banded and randomly distributed into groups of 10-15 birds.

Single Comb White Leghorn adult female chickens were utilized for DDT and endrin chronic and acute exposure

experiments. The birds were housed in individual wire cages 40 cm deep x 45 cm high x 20 cm wide with an inclined floor and kept in an environmental temperature of 21.2°C with a 17 hour per day lighting regime. They were individually wing banded, weighed weekly and randomly distributed into 8-9 birds per group.

V. ELECTROCARDIOGRAM (ECG)

Electrocardiograms were taken on SCWL cockerels before the experiments were terminated. The unanesthetized birds, placed in supine position, were strapped on a V-shaped cradle. The legs were stretched and tied to hooks on the sides of the holder to prevent movement. Needle electrodes were inserted subcutaneously at the base of the right and left wings, and the muscle of the left thigh to form the standard limb leads. Thus, lead I ran from right wing to left wing; lead II from right wing to left leg, and lead III from left wing to left leg. Birds were covered with a piece of cloth and allowed to quiet down prior to the time the electrocardiograms were taken. The ECG's were recorded through the electrodes connected by means of a 5 PG cable to the preamplifier of a Grass Polygraph Model 7 (Grass Instrument Co.; Quincy, Massachusetts). The amplifier was standardized so that 1 mV input gave a pen deflection of 20 mm. The most suitable chart speed was 50 mm per second.

R and S waves from leads II and III were used for calculating the mean electrical axis.

VI. BLOOD PRESSURE AND HEART RATE

Systemic arterial blood pressure and heart rate measurements from birds restrained in a supine position were obtained through a cannulated carotid artery (polyethylene tubing PE 90) using a Statham pressure transducer (Model P 23) and recorded on a Grass polygraph Model 7 (Grass Instrument Co., Quincy, Massachusetts) from either SCWL cockerels under local procaine anesthesia or SCWL adult female chickens anesthetized with sodium phenobarbital (160 mg/Kg body weight). Mean blood pressure was calculated according to Sturkie (1965); mean blood pressure (MBP) = 3/8 pulse pressure (PP) + diastolic blood pressure (DP).

VII. CARDIAC OUTPUT

Cardiac output was measured using a dye-dilution method. A carotid artery was exposed and cannulated by placing a polyethylene tubing connected by an arrangement of 3-way stopcocks either to a pressure transducer or a dye tracer (Gilson Dye Tracer Model DTL; Gilson Medical Electronics, Middleton, Wisconsin). The dye, Cardiogreen (Hynson, Westcott and Dunning, Inc., Baltimore, Maryland), was injected with a 1.0 ml tuberculin syringe into the right jugular vein. The amount of dye injected was 0.1-0.2 ml at a concentration

of 0.2-0.4 mg/ml. A 10 ml lubricated, heparinized syringe was placed on a withdrawal infusion pump Model 950 (Harvard Apparatus Co., Inc., Millis, Massachusetts) and blood was withdrawn at a constant rate of 10 ml/min. Setting a baseline as quickly as possible and while blood was still being drawn through the dye detector unit, Cardiogreen was injected and the dye curve was recorded on an Esterline Angus recorder (Esterline Angus Instrument, Indianapolis, Indiana), at a chart speed of 12 inches/min. After the curve was obtained the blood withdrawal pump was reversed to return the blood to the animal. Calibration of the dye tracer was performed by using an *in vitro* arterial blood sample withdrawn into a heparinized beaker prior to the performance of dye curves. Since blood has a tendency to settle rapidly, which may lead to some erroneous recordings, it was stirred continuously. Ten ml of this blood was used for a control of zero dye concentration. From a stock solution (5 mg/ml) of Cardiogreen, 0.4 ml of dye was diluted in 1.6 ml of aqueous solvent. Four-tenths ml of the latter solution was mixed with 0.4 ml of the solvent to give a concentration of 0.05 mg/0.1 ml. Finally 0.1 ml of this solution was further diluted with 9.9 ml of arterial blood to record the 5 mg/L dilution. Since the dye dilution curve on the descending limb does not reach zero due to recirculation of the dye, it was extrapolated from a replotted downslope curve on two cycle semilog paper.

The area under the curve was calculated with a polar planimeter. Cardiac output (L/min) was calculated according to the following formula:

$$\underset{\text{(L/min)}}{\text{Cardiac output}} = \frac{\text{mg of dye injected}}{\text{area under the curve (mg/L) (min)}}$$

Immediately before measuring cardiac output, heart rate and blood pressure were determined so that these values might be used for calculating stroke volume and peripheral resistance, respectively, according to the following formulas:

$$\underset{\text{(PRU)}}{\text{Peripheral resistance}} = \frac{\text{Mean blood pressure (mm Hg)}}{\text{Cardiac output (ml/min)}}$$

$$\underset{\text{(ml)}}{\text{Stroke volume}} = \frac{\text{Cardiac output (ml/min)}}{\text{Heart rate (beats/min)}}$$

VIII. BLOOD PARAMETERS

Blood samples for analysis were taken in heparinized syringes from the cannulated carotid artery. Packed erythrocyte volume (HCT) was determined by collecting two samples in heparinized capillary tubes. The tubes were centrifuged at 11,500 rpm for five minutes in an International microhematocrit centrifuge. Total erythrocyte counts were made by diluting blood 1:200 with Wiseman's solution (Lucas and Jamroz, 1961) in dilution pipettes and counting in a hemocytometer or by diluting 20λ of blood 1:50,000 with Isoton solution (Scientific Products, Evanston, Illinois) for counting on a Coulter Counter Model B (Coulter Electronics Inc., Hialeah,

Florida). The mean of two determinations was used as an observation. Mean corpuscular volume (MCV) was determined by dividing the packed cell volume by the total erythrocyte count (Wintrobe, 1961). Volumes were converted to μ^3 using appropriate conversion factors. Hemoglobin was determined using the modified Newcomer acid hematin method of Denington and Lucas (1955). Standard curves were made using a prediction equation determined by linear regression using four hemin standards (5, 7.5, 10, and 15 x 10^{-3} mg/ml). These hemin standards corresponded to 5.05, 7.58, 10.11 and 15.16 gm of hemoglobin/100 ml of blood, respectively as determined from a commercial hemoglobin standard (Hycel Hemoglobin control; Hycel, Inc., Houston, Texas). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing hemoglobin concentration by HCT. Blood samples for pH measurement (Corning pH meter Model 12; Corning Scientific Instruments, Medfield, Mass. and Beckman thermomatic constant temperature block) were obtained from the cannulated carotid artery. Blood samples for sodium (Na^+), potassium (K^+) and total thyroxine (T_4) determination were obtained by decapitation while the birds were suspended in an open-end funnel. Blood was collected to heparinized tubes and centrifuged in an International centrifuge (International Equipment Co., Boston, Mass.) at 1500 rpm for 15 minutes. Plasma was withdrawn with a 20 cc syringe,

placed into glass bottles and stored at -15°C until used for analysis.

IX. THYROXINE (T_4) DETERMINATION

Serum thyroxine was measured by the Tetrasorb-125 method (Radio-Pharmaceutical Division, Abbott Laboratories, North Chicago, Illinois) using the principle of "competitive protein binding." There is only a small amount of thyroxine-binding globulin (TBG) in serum, therefore the T_4 binding sites can be readily saturated by adding small amounts of ^{125}I-thyroxine and the fraction which is protein-bound can be determined. If unlabeled T_4 is added, the amount of ^{125}I-labeled bound thyroxine decreases since both the labeled and unlabeled thyroxine compete for the same binding sites. Plasma obtained from blood samples were frozen and stored for periods of several weeks. Before use, the plasma samples were thawed and brought to room temperature. One ml of plasma was placed in a centrifuge tube and 2 ml of 95% ethanol were added. The solution was mixed for a few seconds by means of a Vortex mixer and then centrifuged at 1000 g for 20 minutes. One 0.6 ml aliquot of the supernatant was transferred to a polypropylene test tube. A stream of clean air was used to evaporate the liquid in the tubes to dryness while the tubes were immersed in a warm-water bath (30°C). Then 1 ml of TBG-Thyroxine ^{125}I reagent solution was added to the

bottom of each tube and gently shaken for a few moments to help dissolve the film which was formed during evaporation. The tubes were allowed to equilibrate at room temperature for ten minutes so that equilibration between labeled and unlabeled thyroxine and TBG molecules might be reached. Then the tubes were placed in an ice bath (0° to 4° C) for five minutes. After this five minute period one Tetrasorb resin-sponge was placed in each tube and all air was expressed from the resin-sponge by means of the Abbott plastic plunger. The plunger was rinsed with water between uses. After 30 minutes of the incubation period an initial count (IC) was made from each tube. The incubation period was ended after 60 minutes by adding cool glass-distilled water (about 10 ml) to each tube. The water was removed by compressing the sponge 3-4 times with the Abbott aspirator. Then each tube was washed three times with cool glass-distilled water. Finally the remaining radioactivity of the sponge was counted (FC) using a well-type scintillation counter (Nuclear Chicago, Model Ds-5) and analyzer-scaler (Nuclear Chicago, Model 8725). The percentage ^{125}I-Thyroxine uptake by the resin-sponge was calculated according to the following formula:

$$\%\ ^{125}\text{I-thyroxine uptake} = \frac{\text{FC (CPM)} - \text{background (CPM)}}{\text{IC (CPM)} - \text{background (CPM)}} \times 100$$

A standard curve was made from a standard stock solution prepared from crystalline free thyroxine purified from monosodium thyroxine pentahydrate (Baxter Laboratories, Morton Grove, Illinois). Ten mg of free T_4 was dissolved in 95% ethanol and diluted to a concentration of 5 µg/ml. The standard curve was obtained using three thyroxine standards (2.5, 5 and 10 µg/100 ml) and the T_4 values were calculated by using the "linear regression equation" (Li, 1964).

X. SODIUM (Na^+) AND POTASSIUM (K^+) DETERMINATION

The concentrations of Na^+ and K^+ in the plasma and pericardial fluid samples were determined by flame emission spectrophotometry. The method is based on the ability of the electrons of certain neutral atoms (such as sodium and potassium) to be raised from the passive state to an excited state by heat. In returning to the passive state, the excited electrons are capable of emitting radiation of a specific wave-length which is specific for each atom, being the intensity of radiation equivalent to the number of atoms excited. Thus the radiation emitted can be determined.

Plasma obtained from blood samples and pericardial fluid were frozen (-15°C) and stored for periods of several weeks. Before use, the plasma and pericardial fluid samples were thawed and brought to room temperature. They were diluted 1:100 and 1:10 with deionized distilled water for

Na^+ and K^+, respectively, and determinations were made using a Jarrel-Ash Model 82-156 (Jarrel-Ash Company, Waltham, Mass.) atomic absorption spectrophotometer equipped with a Hetco total consumption burner. Samples were aspirated into a air-hydrogen flame, and a wavelength of 5889A° was used for Na^+ and 7555A° for K^+, respectively. The emission signal was recorded in a chart recorder Model SRL (B. E. Sargent and Co., Skokie, Ill.) that was connected to the spectrophotometer through a signal modifying unit. A standard curve was made for each run using a standard solution of sodium chloride (0.2, 0.8, 1.4 and 2.0 mEq/L) and potassium chloride (0.12, 0.48, 0.84 and 1.30 mEq/L) and the unknown values were determined from the quadratic equation obtained by multiple linear regression.

XI. ACUTE EXPOSURE EXPERIMENTS

In 16 experiments, SCWL adult females weighing from 1.3 to 2.0 Kg were anesthetized intravenously (brachial vein) with sodium phenobarbital (160 mg/Kg body wt.). A femoral or brachial vein was isolated and cannulated with a polyethylene tubing (PE 90) for drug administration. An acute dose (8 mg/Kg body wt.) of the insecticide endrin (Tech. 95%; City Chemical Corporation, New York) dissolved in 95% ethanol was infused (Harvard Infusion pump) at 0.05-0.1 ml/min. (20 mg/ml solution concentration) through the

cannulated vein. Alcohol blank infusions at the rate and volume used with endrin were also administered. Systemic arterial pressure and heart rate measurements were obtained as described previously. After infusion of endrin, heart rate was measured periodically until the cardiac effect was pronounced. Then, an atropine injection (0.2 mg) was given intravenously. Blood samples for hemoglobin determination, HCT and total erythrocyte counts were taken with heparinized syringes from the cannulated carotid artery and treated in the same way as described previously. In a few cases, a subsequent dose of phenobarbital was given to alleviate convulsions after endrin administration. All the above parameters were recorded at 15-minute intervals for 60 minutes after endrin infusion in those cases where the animals remained alive. At that time the experiments were terminated.

XII. PERICARDIAL FLUID

Hydropericardium volume was determined by inserting a 20 gauge needle attached to a 2.5 ml syringe into the pericardium and withdrawing the fluid which was then measured to the nearest 0.1 ml. Pericardial fluid was placed into glass bottles and stored at -15°C until used for analysis.

XIII. ORGANS

The birds were sacrificed and various organs obtained. Organs were dissected free of fat and surrounding tissue and weighed on a Mettler scale Model P 1210 (Mettler Instrument Co., Heightstown, New Jersey) to the nearest 0.1 gram. The organs collected were heart, liver, spleen, thyroid, testes and comb. To insure greater accuracy, thyroids were weighed on a Roller-Smith precision balance (Fisher Scientific Co., Pittsburgh, Pennsylvania) to the nearest 0.1 mg. Testes and thyroids were kept in 10% neutral buffered formalin solution. They were sectioned and stained routinely with hematoxylin and eosin stain (Lillie, 1957) for histological evaluation.

XIV. BODY WEIGHT

Body weights were measured before and throughout the experiments to the nearest gram using a direct recording scale (Toledo Scale Co., Toledo, Ohio).

XV. STATISTICAL ANALYSES

All data obtained were statistically analyzed by either Analysis of Variance or row by column Chi-square analysis. Parameters showing significant effects by Analysis of Variance were further tested by the New Multiple Range procedure of Duncan (1955). Statistical analyses (Analysis of Variance and Duncan's test) were programmed on an Olivetti-Underwood

Programma 101 desk computer. The data from the PCB experiment 4 (pair-fed experiment) were analyzed by factorial (2-way) Analysis of Variance using a least squares procedure. Scheffe's test (Snedecor and Cochran, 1967) was used for mean separation of significant effects. These statistical analyses were carried out in a CDC 6500 computer.

RESULTS AND DISCUSSION

I. CHRONIC EXPOSURE EXPERIMENTS WITH DDT AND ENDRIN

If a pesticide or any other compound is incorporated into the diet, its effect on any particular parameter of an animal is going to depend upon the length of time that the compound is fed, the dietary level fed and the amount of feed that the animal ingests daily. Feed consumption or any other related parameter was not the main goal of this study, therefore the pesticide levels administered to chickens were those reported in the literature to be most suitable for bringing about biological changes after being fed for a certain period of time.

In Experiment 1, six groups of nine Single Comb White Leghorn mature females each were fed DDT at a rate of 0, 400 and 800 ppm and endrin at 0, 8 and 16 ppm for a period of eight weeks. In Experiment 2, six groups of eight birds each were fed 0, 1000 or 2000 ppm DDT, or endrin at a rate of 0, 10 or 20 ppm during six weeks. Average body weights as well as various organ weights are reported in Tables 3 and 4 showing the effect of the various levels of DDT and endrin when they were incorporated into the diet of chickens. Tables 5 and 6 show the organ weights expressed in terms of

containing DDT and endrin on body, heart, liver and spleen

Table 3. The effect of feeding rations containing DDT and endrin on body, heart, liver and spleen weights of SCWL adult females.

Treatment[a]	Parameter			
	Body wt. (Kg)	Heart wt. (gm)	Liver wt. (gm)	Spleen wt. (gm)
DDT control	(9) 1.8±0.05[b]	(9) 7.6±0.28	(9) 37.8±1.9[c] $_m$	(9) 1.9±0.20
DDT 500 ppm	(9) 2.0±0.11	(9) 9.6±1.01	(9) 37.3±1.8 $_m$	(9) 1.9±0.30
DDT 1000 ppm	(9) 1.9±0.09	(9) 9.0±0.69	(9) 44.3±1.7 $_n$	(9) 2.0±0.32
Endrin control	(9) 1.9±0.06 $_m$	(9) 8.4±0.42	(9) 38.9±3.0	(9) 1.8±0.20
Endrin 8 ppm	(9) 1.9±0.15 $_m$	(9) 8.9±0.78	(9) 35.3±2.7	(9) 1.5±0.11
Endrin 16 ppm	(7) 1.6±0.07 $_n$	(7) 9.0±0.99	(7) 36.1±2.8	(7) 1.9±0.20

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different, m, n, ($P<0.05$)

Table 4. The effect of feeding rations containing DDT and endrin on body, heart, liver and spleen weights

Table 4. The effect of feeding rations containing DDT and endrin on body, heart, liver and spleen weights of SCWL adult females.

Treatment[a]	Parameter			
	Body wt. (Kg)	Heart wt. (gm)	Liver wt. (gm)	Spleen wt. (gm)
DDT control	(7) 1.6±0.08[b]	(7) 7.0±0.39	(7) 46.7±4.40	(7) 1.8±0.34
DDT 1000 ppm	(7) 1.5±0.13	(7) 6.8±0.40	(7) 37.9±4.32	(7) 2.2±0.42
DDT 2000 ppm	(5) 1.5±0.05	(5) 7.1±0.62	(5) 35.8±2.70	(5) 1.4±0.11
Endrin control	(7) 1.7±0.08	(7) 7.9±0.74	(7) 47.8±3.60	(7) 1.8±0.22
Endrin 10 ppm	(7) 1.7±0.14	(7) 7.7±0.52	(7) 40.1±4.52	(7) 1.5±0.20
Endrin 20 ppm	(6) 1.6±0.09	(6) 8.0±0.73	(6) 32.7±3.81	(6) 1.5±0.20

[a] Rations fed up to 6 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

Table 3: The effect of feeding rations containing DDT and endrin on heart, liver and spleen expressed as

Table 5. The effect of feeding rations containing DDT and endrin on heart, liver and spleen expressed as percentage of body weight of SCWL adult females.

Treatment[a]	Parameter		
	Heart	Liver	Spleen
DDT control	(9) 0.4±0.02[b]	(9) 2.1±0.12[c] $_{mn}$	(9) 0.11±0.010
DDT 500 ppm	(9) 0.5±0.03	(9) 1.9±0.10 $_{n}$	(9) 0.09±0.007
DDT 1000 ppm	(9) 0.5±0.02	(9) 2.4±0.13 $_{m}$	(9) 0.11±0.017
Endrin control	(9) 0.4±0.02	(9) 2.0±0.12	(9) 0.09±0.006 $_{m}$
Endrin 8 ppm	(9) 0.5±0.04	(9) 1.9±0.15	(9) 0.08±0.011 $_{m}$
Endrin 16 ppm	(7) 0.6±0.06	(7) 2.3±0.17	(7) 0.12±0.007 $_{n}$

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$)

Table 6. The effect of feeding rations containing DDT and endrin on heart, liver and spleen expressed as percentage of body weight of SCWL adult females.

Treatment[a]	Parameter		
	Heart	Liver	Spleen
DDT control	(7) 0.4±0.01[b]	(7) 2.9±0.21	(7) 0.11±0.020
DDT 1000 ppm	(7) 0.5±0.02	(7) 2.6±0.14	(7) 0.20±0.028
DDT 2000 ppm	(5) 0.5±0.03	(5) 2.4±0.13	(5) 0.10±0.008
Endrin control	(7) 0.5±0.04	(7) $2.9\pm0.20_{m}$[c]	(7) 0.11±0.010
Endrin 10 ppm	(7) 0.5±0.03	(7) $2.4\pm0.10_{n}$	(7) 0.09±0.004
Endrin 20 ppm	(6) 0.5±0.03	(6) $1.9\pm0.18_{n}$	(6) 0.09±0.010

[a] Rations fed up to 6 weeks

[b] Data are repotted as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$)

percentage of the total body weight. DDT did not produce any significant change in body weight at any level fed. On the other hand, endrin did decrease body weight significantly ($P < 0.05$) at 16 ppm for eight weeks when compared with the control values. However, in experiment 2 where endrin was fed at higher levels (20 ppm) for six weeks, it did not produce any significant change in this particular parameter. This fact could be explained due to the shorter time that the highest level was fed, indicating that time is an important factor in influencing the toxic effects of a compound as was stated previously.

Liver weight was significantly higher from chickens fed DDT at 1000 ppm in experiment 1 than those from chickens exposed to control diets. Likewise, liver weight as percentage of body weight was significantly lower from birds fed DDT at 500 ppm. At higher levels, DDT did not alter liver weight. It is possible that the increase in liver weight might be related to stimulation of drug metabolism. However, since no hepatic test was performed no further conclusions can be drawn. Hart and Fouts (1965) have reported these relationships previously. Cecil et al. (1973), have shown that DDT increased liver weight in rats and Japanese quail. Endrin did not affect liver weight at any level fed; however, when liver weights were expressed as percentage of body weight they were significantly lower in experiment 2 at

10 and 20 ppm compared with the values from control birds. Treon et al. (1955), found that dogs fed endrin at 8 ppm for 24 weeks had enlarged livers and rats given 5-25 ppm for two years had significantly increased liver weight when expressed as percentage of body weight. This difference in response could be due to the different length of time that endrin was fed and/or because of species difference. Spleen weight was not affected by incorporation of DDT or endrin in the diet, but as percentage of body weight it was significantly higher in endrin-treated birds at 16 ppm than in controls. The data show, however, that actual spleen weights were not affected; therefore, the percentage change was caused by the body weight reduction.

It has been established that some pesticide materials whether incorporated in the diet or given by injection, may affect hematological and cardiovascular parameters in different animals. The chronic effects of DDT and endrin on heart rate and mean arterial blood pressure are shown in Tables 7 and 8. It is evident from the tables that DDT and endrin did not cause any significant effect on these parameters when they were compared with values from control groups at any level fed.

The effect of feeding various levels of DDT and endrin on HCT, hemoglobin concentration and arterial blood pH in the adult female chicken is reported in Tables 9 and 10.

Table 7. The effect of feeding rations containing DDT and endrin on heart rate and mean blood pressure of SCWL adult females.

Treatment[a]	Parameter	
	Heart rate (beats/min)	Mean blood pressure[b] (mm/Hg)
DDT control	(9) 319±9.3[c]	(9) 103±4.2
DDT,500 ppm	(9) 303±15.4	(9) 100±9.5
DDT 1000 ppm	(8) 285±12.1	(8) 97 ±5.4
Endrin control	(9) 343±7.3	(9) 104±4.5
Endrin 8 ppm	(8) 329±12.9	(8) 103±4.9
Endrin 16 ppm	(7) 344±8.8	(7) 109±10.9

[a]Rations fed up to 8 weeks

[b]Mean blood pressure: MBP = 3/8 PP + DP

[c]Data are reported as (No. of birds) Mean ± Standard Error

Table 8. The effect of feeding rations containing DDT and endrin on heart rate and arterial blood pressure of SCWL adult females.

Treatment[a]	Parameter	
	Heart rate (beats/min)	Mean blood pressure[b] (mm Hg)
DDT control	(6) 340±11.0[c]	(6) 105±5.4
DDT 1000 ppm	(7) 311±12.6	(7) 100±5.4
DDT 2000 ppm	(5) 340±25.9	(5) 101±6.6
Endrin control	(7) 334±11.5	(7) 90 ±4.9
Endrin 10 ppm	(7) 353±9.0	(7) 95 ±5.1
Endrin 20 ppm	(6) 350±15.3	(6) 107±5.2

[a]Rations fed up to 6 weeks

[b]Mean blood pressure: MBP = 3/8 PP + DP

[c]Data are reported as (No. of birds) Mean ± Standard Error

Table 9. The effect of feeding rations containing DDT and endrin on packed erythrocyte volume, hemoglobin concentration and arterial blood pH of SCWL adult females.

Treatment[a]	Parameter		
	Packed erythrocyte volume (%)	Hemoglobin conc. (gm/100 ml)	Arterial blood pH
DDT control	(9) 27.4±0.87[b]	(9) 9.0±0.24	(9) 7.51±0.020
DDT 500 ppm	(9) 27.1±0.59	(9) 9.2±0.26	(9) 7.47±0.011
DDT 1000 ppm	(8) 25.0±0.95	(8) 9.0±0.32	(8) 7.51±0.014
Endrin control	(9) 26.9±0.64[c]$_m$	(9) 9.3±0.21$_x$	(9) 7.51±0.019
Endrin 8 ppm	(8) 27.6±1.03$_m$	(8) 9.3±0.39$_x$	(8) 7.53±0.030
Endrin 16 ppm	(7) 31.1±1.11$_n$	(7)10.8±0.39$_y$	(7) 7.50±0.020

[a]Rations fed up to 8 weeks

[b]Data are reported as (No. of birds) Mean ± Standard Error

[c]Means having different subscripts are significantly different; m, n ($P<0.05$) x, y ($P<0.01$)

Table 10. The effect of feeding rations containing DDT and endrin on packed erythrocyte volume, hemoglobin concentration and arterial blood pH of SCWL adult females.

Treatment[a]	Parameter		
	Packed erythrocyte volume (%)	Hemoglobin conc. (gm/100 ml)	Arterial blood pH
DDT control	(6) $26.0 \pm 1.2^{bc}_{m}$	(6) $9.5 \pm 0.39_{m}$	(6) 7.49 ± 0.03
DDT 1000 ppm	(7) $26.2 \pm 1.7_{m}$	(7) $9.3 \pm 0.64_{m}$	(7) 7.46 ± 0.02
DDT 2000 ppm	(5) $21.1 \pm 0.7_{n}$	(5) $7.1 \pm 0.37_{n}$	(4) 7.46 ± 0.01
Endrin control	(6) $27.4 \pm 0.4_{m}$	(6) $9.9 \pm 0.18_{m}$	(6) 7.51 ± 0.01
Endrin 10 ppm	(7) $28.3 \pm 1.2_{m}$	(7) $10.2 \pm 0.37_{m}$	(7) 7.49 ± 0.01
Endrin 20 ppm	(6) $32.2 \pm 1.8_{n}$	(6) $11.3 \pm 0.49_{n}$	(6) 7.52 ± 0.01

[a] Rations fed up to 6 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n, ($P<0.05$)

A significant decrease ($P < 0.05$) in HCT values was observed in female chickens after receiving DDT in the diet at a rate of 2000 ppm. In experiment 1 the trend was the same with the highest level even though it was not significantly different. A significant decrease ($P < 0.05$) in hemoglobin concentration was also found in chickens receiving the highest level in experiment 2. These results are in agreement with those reported by Cameron and Burgess (1945) who showed that chronic doses of DDT decreased hemoglobin concentration in rabbits and those from Ernst and Ringer (1968) who found a decrease in HCT and the total erythrocyte count of the blood of mature male Japanese quail. In addition, a decrease in total erythrocyte number was observed even though this parameter was not quantitated. Therefore, it is possible that the decrease in HCT after DDT ingestion was due to a decrease in total number of erythrocytes, rather than an increase in blood volume and/or a direct detrimental effect in erythropoiesis or indirectly by inhibiting normal production of erythropoietin.

In contrast, endrin caused a significant increase ($P < 0.05$) in the packed cell volume of the mature female chicken in both experiments with the highest levels fed (16 and 20 ppm, respectively). A significant change in the same direction was observed in total hemoglobin concentration with these same levels. A similar increase in HCT was

observed by Emerson (1964) in dogs after acute endrin poisoning. The packed cell volume may increase because of an increase in erythrocyte concentration, and/or an increase in cell size. Concentration of red blood cells can rise due to an increase in production and release of red blood cells into the circulation, a decrease in plasma volume or both. In his study, Emerson (1965) indicated that erythrocyte expulsion from spleen storage was the main factor responsible for the increase in HCT after endrin administration in dogs. However, this possibility is very unlikely in chickens. The degree of contractility in the mammalian spleen is enhanced because of the thick muscular capsule and prominent trabeculae of the organ, whereas, in birds the spleen has a thin capsule with scarce muscle fibers, therefore its ability to contract is highly diminished (Sturkie, 1965). Another possibility to explain this change in HCT would be a decrease in plasma volume, since a significant decrease in body weight was observed. However, birds receiving endrin did not exhibit fluid loss as observed at gross post-mortem examination. A stimulatory action of endrin in normal production of erythropoietin and in turn enhancing erythropoiesis can not be disregarded.

Neither DDT nor endrin produced any change in arterial blood pH at any level fed (Tables 9 and 10).

II. ACUTE EXPOSURE EXPERIMENTS

The effects that acute doses of infused endrin may have on the cardiovascular system of the mature female chicken were investigated. In this study, intravenous endrin administration to SCWL adult females resulted in differing symptoms. Slight tremors appeared first in these birds, followed in most cases by severe tonic and clonic convulsions manifested by a burst of strong wing-beat convulsions which usually started within 5 to 10 minutes after endrin infusion was ended. In addition, mild tremors involving the head and legs also occurred accompanied by throat sounds. Most birds showed mucoid salivation and exaggerated response to sudden sound or tactile stimuli. All the previous symptoms lasted until the bird died. Because of the severe consulsions which developed as a result of endrin infusion it was necessary to give subsequent injections of sodium phenobarbital (80-100 mg/inj.) in some cases. Endrin administration may have a direct action on the spinal cord and/or motor cortex as reflected by the presence of convulsions and excitability. Increased salivation indicated a parasympathetic stimulation. Similar symptoms have been reported in the literature in different animals, including man, after exposure to various chlorinated hydrocarbon insecticides (Black and Ecobichon, 1971; Harr et al., 1970; Garretson and Curley, 1969; Emerson et al., 1964; Treon et al., 1955).

Figure 1 shows the effect of endrin infusion on heart rate. A severe decrease in heart rate developed ($P < 0.01$) after endrin administration. Only 2 out of 16 preparations did not show this dramatic effect even though a mild bradycardia (less than 45 beats/min. difference compared to control values) was elicited in both cases. In a total of 16 preparations analyzed, it took 6 to 34 minutes (average 20 ± 2.1) for this bradycardia to reach its lowest value (186 ± 9.2). When this cardiac effect was pronounced, atropine was injected intravenously. Atropine injection resulted in an immediate return of heart rate towards or above control values (342 ± 11.3 beats/min.) which lasted until the end of the experiment or the bird's death. The mean values of arterial blood pressure before, during and after endrin infusion are shown in Figure 2. In 15 preparations studied, mean systemic arterial blood pressure significantly ($P < 0.01$) increased, reaching a maximum of 150 ± 3.8 mm Hg after an average time of 15 ± 3.9 minutes post-endrin administration but decreased (except two birds) toward or below control values (110 ± 4.1 mm of Hg) some 17 minutes later. The fact that bradycardia observed after endrin infusion occurred following hypertension by an average time of five minutes (11 out of 15 cases), would indicate that the hypertension observed was not a reflex response to the bradycardia. Therefore, it is possible that the bradycardia

Figure 1. Effect of endrin infusion (8 mg/Kg body weight) on the heart rate of SCWL adult female; n = number of birds. Standard error of the mean is indicated.

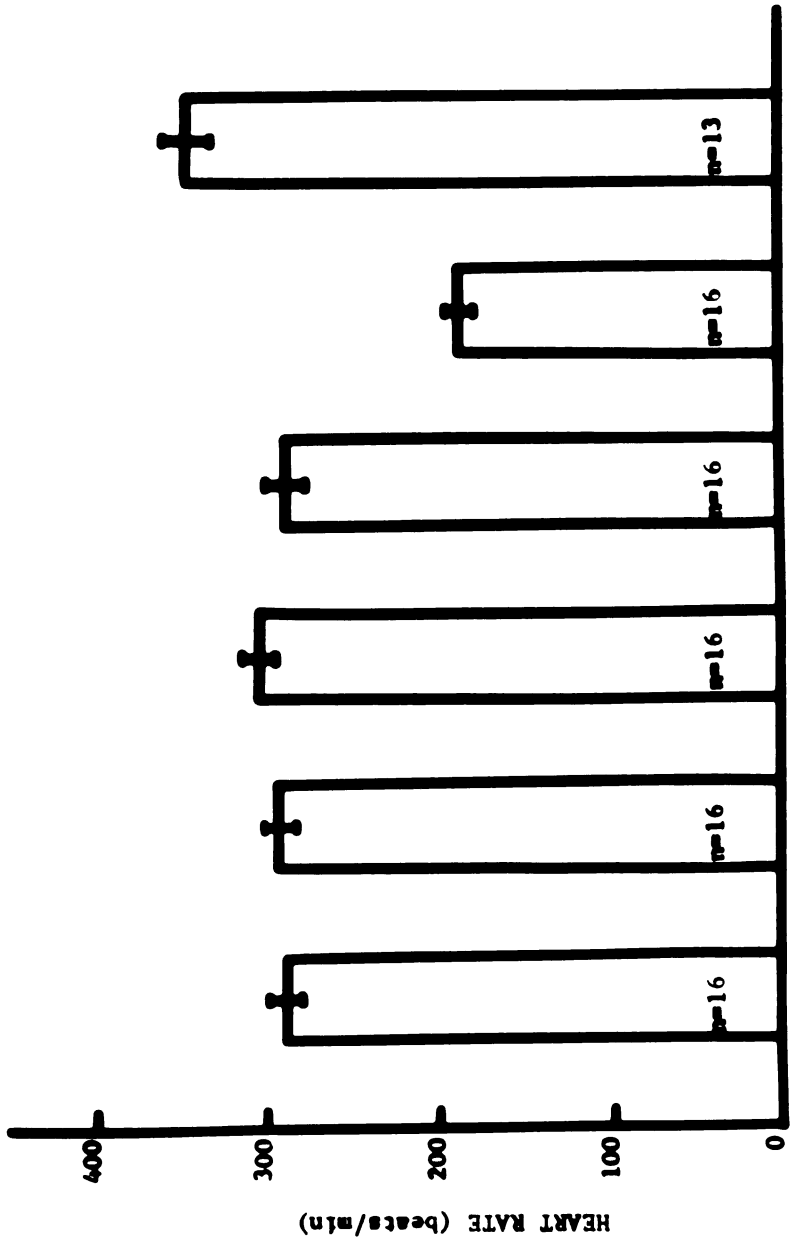
HEART RATE (beats/min)
400
300
200
100
0
n=16
n=16
n=16
n=16
n=16
n=13
Before Alcohol
After Alcohol
Before Endrin
After Endrin
Maximum Bradycardia
After Atropine

Figure 1

Figure 2. Effect of endrin infusion (8 mg/Kg body weight) on the mean blood pressure of SCWL adult female; n = number of birds. Standard error of the mean is indicated.

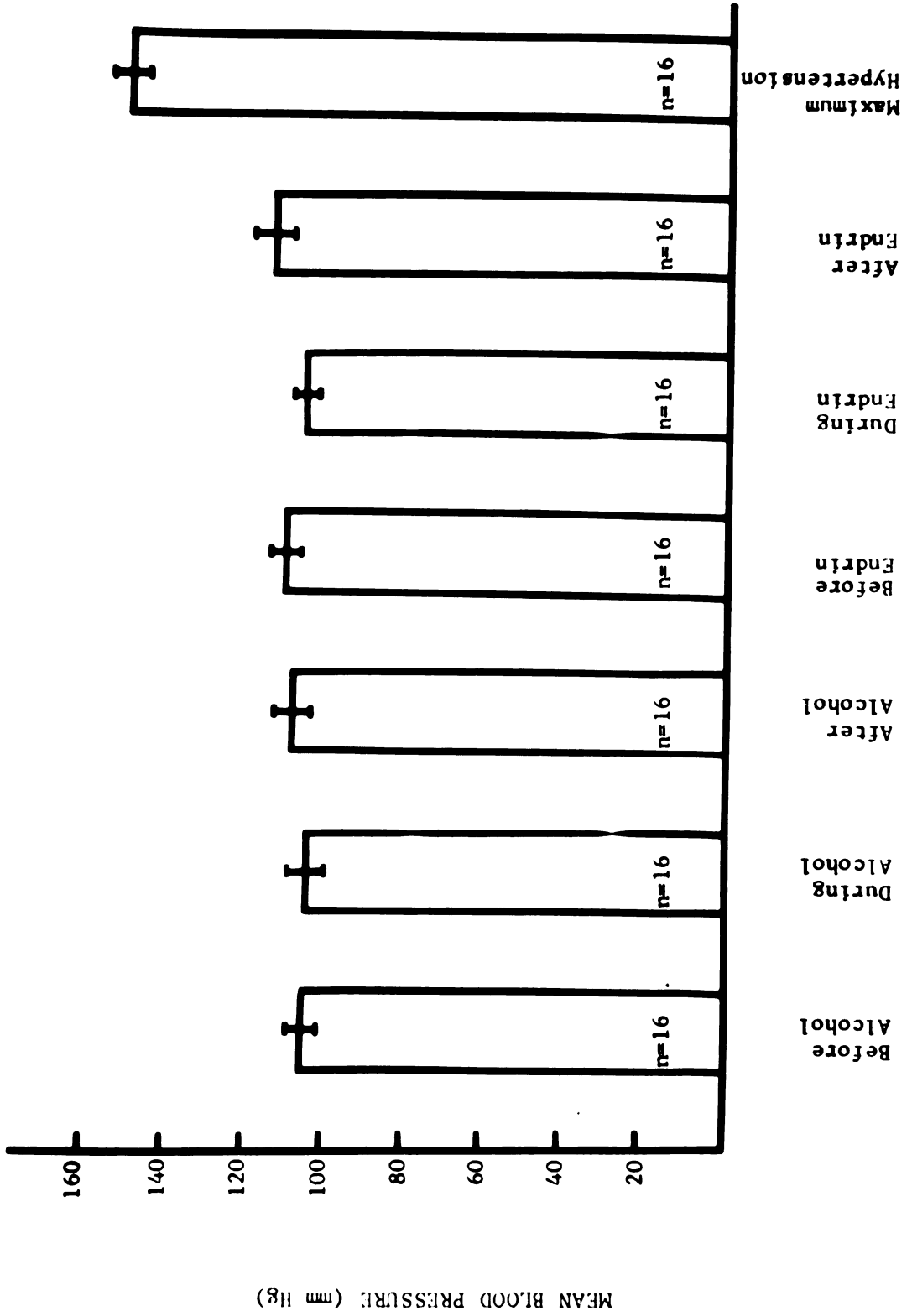
MEAN BLOOD PRESSURE (mm Hg)
160
140
120
100
80
60
40
20
n=16
Before Alcohol
During Alcohol
After Alcohol
Before Endrin
During Endrin
After Endrin
Maximum Hypertension

Figure 2

obser

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observed in these birds might occur as a result of parasympathetic stimulation whereas the hypertension could be due to sympathetic action. In four cases hypertension and bradycardia developed simultaneously. Whether the bradycardia observed in these four birds was in response to the hypertension as a reflex action or as direct stimulation of the parasympathetic and sympathetic systems is not clear. Emerson et al. (1964), observed that hypotension and bradycardia developed simultaneously or bradycardia was preceded by hypertension after acute endrin poisoning in dogs. Since consistent findings after endrin administration were convulsion, hypertension, excessive salivation and decrease in heart rate, these authors suggested that sympathetic and parasympathetic nervous systems were hyperactive. Since the same type of symptoms were observed in birds, similar mechanisms may be acting and would account for the changes observed after endrin administration. No significant differences were observed, before and after (15-minute interval) endrin infusion, in total erythrocyte count, packed erythrocyte volume and hemoglobin concentration in 16 preparations analyzed (Figure 3). Alcohol blank infusions did not have any cardiovascular or hematological effects on the parameters studied.

Figure 3. Effect of endrin infusion (8 mg/Kg body weight) on the hemoglobin concentration, hematocrit and total erythrocyte concentration of SCWL adult female. Mean values ± S. E. Number of animals indicated in upper frame.

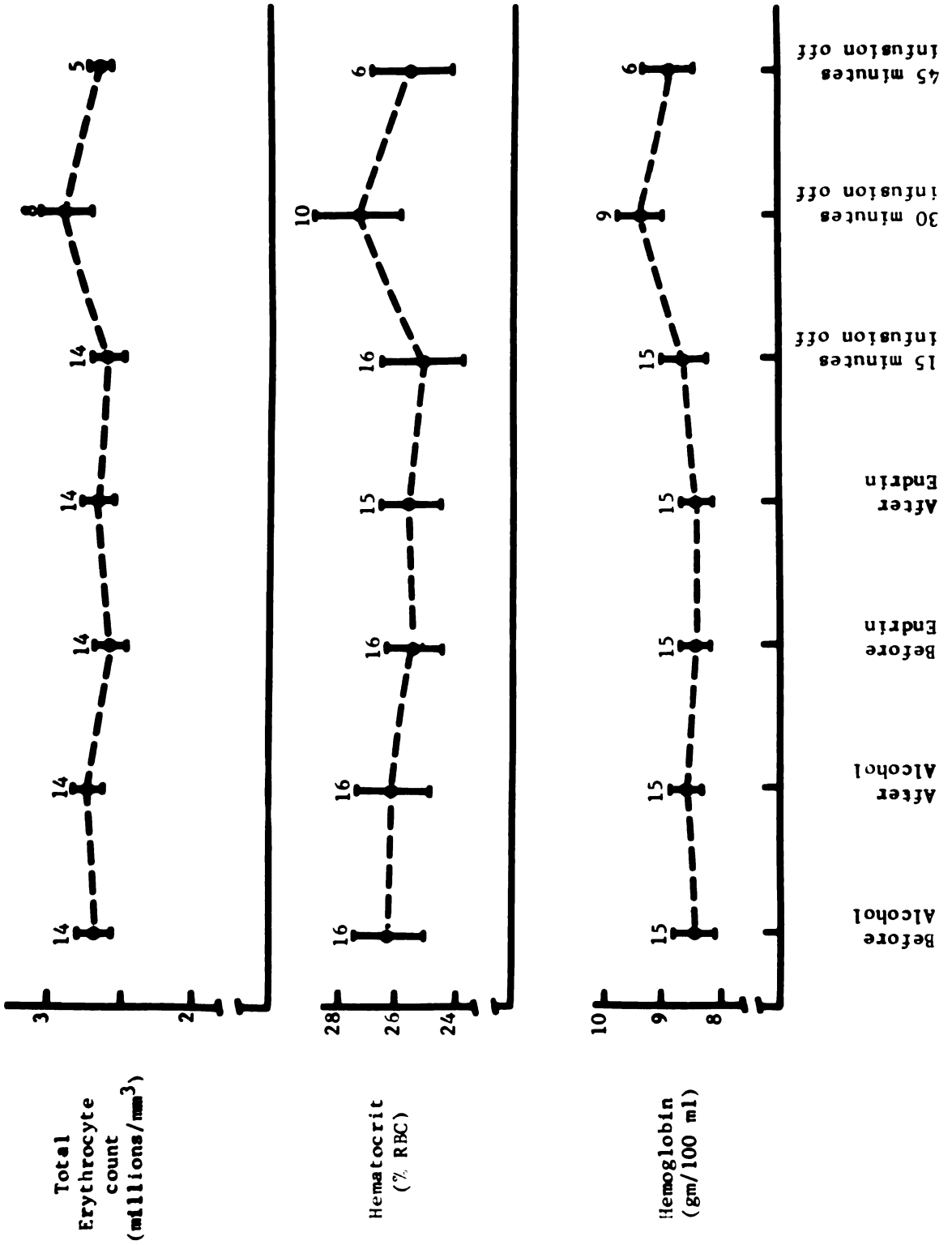
Total Erythrocyte count (millions/mm3)
3
2
14
14
14
14
14
8
5
Hematocrit (% RBC)
28
26
24
16
16
16
15
16
10
6
Hemoglobin (gm/100 ml)
10
9
8
15
15
15
15
15
9
6
Before Alcohol
After Alcohol
Before Endrin
After Endrin
15 minutes infusion off
30 minutes infusion off
45 minutes infusion off

Figure 3

III. CHRONIC EXPOSURE EXPERIMENTS WITH CHLORINATED BIPHENYLS

A. Experimental design

Experiment 1.--In trial 1, rations containing 25, 50 and 100 ppm of Aroclor 1242 were fed to three groups of 12 one-day-old Single Comb White Leghorn cockerels maintained at the M.S.U. Poultry Science Research and Teaching Center. In a second trial, the same Aroclor used in trial 1 was added to rations to give 50, 100 and 200 ppm. All groups were terminated at eight weeks except in trial two when the group fed 200 ppm was sacrificed at four weeks due to high mortality; a corresponding number of controls were sacrificed at this time.

Experiment 2.--Rations containing 50, 100 and 150 ppm of Aroclor 1221, 1254 and 1260 were given to one-day-old commercial (De Kalb Strain) SCWL cockerels. The experiment was terminated when birds were nine weeks of age.

Experiment 3.--Three groups of 15 one-day-old SCWL cockerels (De Kalb Strain) were fed 0 and 100 ppm Aroclor 1242 and 1254. The experiment was terminated at eight weeks.

Experiment 4.--It was noticed in previous studies that a drop in feed consumption was a consistent symptom of PCB toxicity, therefore a pair-fed experiment was set up to determine whether feed restriction may or may not alter some of the parameters under investigation. Ten one-day-old

SCWL cockerels (De Kalb Strain) were alloted for each of the three replicates in each group. Details for the feeding regimen have been described in Section III of Materials and Methods. The experimental group received 100 ppm Aroclor 1242 while the pair-fed group received the basal ration with no PCB. A third group was fed the basal ration ad libitum. The experiment was terminated at eight weeks.

Experiment 5.--Two groups of one-day-old SCWL cockerels (De Kalb Strain) were fed PCB 1016 at levels of 0 (15 birds) and 150 ppm (12 birds). Birds were sacrificed at nine weeks of age.

B. Mortality and Hydropericardium

Most of the toxicological symptoms reported in the literature as a result of PCB ingestion were observed in experiments 1-4. Mortality was low with low levels (25-50 ppm), becoming more severe when levels in the diet were raised, being 41% with 150 ppm PCB 1254 and 43% and 53% with 100 and 200 ppm PCB 1242, respectively. No mortality due to treatment was observed in birds which were fed Aroclor 1016. Flick et al. (1965), reported no deaths when SCWL cockerels were fed diets containing 200 ppm PCB 1242 for three weeks and only 4 out of 24 chickens on a diet containing 400 ppm of the same PCB for an equal length of time. Mortality was as severe with PCB 1248 at 50 ppm in the diet of cockerels as with three times as much fed for 4½ to 5 weeks (Rehfeld

et al., 1971). Total mortality was observed between 6 and 55 days after feeding 2000 ppm PCB (60% chlorinated) in the diet of Japanese quail (Koeman et al., 1969). The difference in length of time, levels fed and percent of clorination may account for the different mortality rates reported in this study and those which have appeared in the literature. General edema and hydropericardium was observed in birds fed diets containing as low as 50 ppm PCB's 1242 and 1254 (Table 11). In experiment 4, 100% of the chickens treated with 100 ppm PCB 1242 showed hydropericardium. Pericardial fluid ranged from 1.8 to 27.0 ml with an average of 10.3 ml. Only three out of 15 chickens fed Aroclor 1016 showed hydropericardium (> 1.0 ml of pericardial fluid). Edema formation and hydropericardium have been reported by other investigators when various PCB's were fed (McCune et al., 1962; Flick et al., 1965; Vos and Koeman, 1970; Rehfeld et al., 1971).

C. Body and organ weights

Body and various organ weights were measured in experiments in which different levels of PCB's varying in percentage of chlorine were incorporated into the rations of SCWL cockerels. These results are shown in Tables 12, 13, 14, 15 and 16. It is apparent from the data presented in these tables that birds receiving PCB's 1242 at 100 ppm and 1254 at levels as low as 50 ppm markedly decreased body

Table 11. Percentage of chickens exhibiting hydropericardium[a] when fed various Aroclors from 1 day of age.

Aroclor	Treatment period (weeks)[b]	Dietary level (ppm) 0	25	50	100	150	χ^2
1221	9	0% (11)[c]	---	0% (11)	0% (10)	0% (11)	NS
1242	8 (trial 1)	0% (12)	0% (12)	20% (10)	62% (8)	---	P<0.001
1242	8 (trial 2)	0% (10)	---	25% (12)	80% (10)	---	P<0.001
1254	9	0% (12)	---	8% (11)	50% (8)	57% (7)	P<0.05
1260	9	0% (12)	---	0% (12)	9% (12)	8% (12)	NS

[a]Hydropericardium taken as >1.0 ml pericardial fluid

[b]Average at sacrifice

[c](No. of birds)

Table 12. The effect of feeding rations containing Aroclor 1242 on body, heart, liver, spleen and testes weight of SCWL cockerels.

Parameter	Treatment[a] (ppm) 0	25	50	100
Body wt (gm)	(12) 522 ± 14.4[b]	(12) 513 ± 17.7	(10) 531 ± 15.5	(8) 501 ± 35.6
Heart wt (gm)	(12) 3.1 ± 0.16	(12) 2.9 ± 0.09	(10) 3.0 ± 0.20	(8) 3.6 ± 0.26
Heart (% b. wt)	(12) 0.6 ± 0.22[c] $_{m}$	(12) 0.6 ± 0.01 $_{m}$	(10) 0.6 ± 0.02 $_{m}$	(8) 0.7 ± 0.07 $_{n}$
Liver wt (gm)	(12) 20.6 ± 0.96 $_{m}$	(12) 25.6 ± 1.51 $_{n}$	(10) 24.5 ± 1.07 $_{n}$	(8) 19.1 ± 1.29 $_{m}$
Liver (% b. wt)	(12) 3.9 ± 0.15 $_{xy}$	(12) 5.0 ± 0.22 $_{x}$	(10) 4.6 ± 0.10 $_{x}$	(8) 3.7 ± 0.21 $_{y}$
Spleen wt (gm)	(12) 1.4 ± 0.14 $_{m}$	(12) 1.4 ± 0.09 $_{m}$	(10) 0.9 ± 0.08 $_{n}$	(8) 1.0 ± 0.18 $_{n}$
Spleen (% b. wt)	(12) 0.26± 0.02	(12) 0.22± 0.01	(10) 0.17± 0.01	(8) 0.26± 0.06
Testes wt (gm)	(12) 0.3 ± 0.04	(12) 0.3 ± 0.03	(10) 0.2 ± 0.02	(8) 0.2 ± 0.06
Testes (% b. wt)	(12) 0.06± 0.010	(12) 0.06± 0.007	(10) 0.04± 0.004	(8) 0.04± 0.012

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 13. The effect of feeding rations containing Aroclor 1242 on body, heart, liver, spleen and testes weight of SCWL cockerels.

Parameter	Dietary level (ppm)[a]		
	0	50	100
Body wt (gm)	(10) $648 \pm 32.6^{bc}_{x}$	(12) $601 \pm 21.0_{xy}$	(10) $500 \pm 27.6_{y}$
Heart wt (gm)	(10) 3.4 ± 0.23	(12) 3.2 ± 0.14	(10) 3.2 ± 0.26
Heart (% b.wt)	(10) $0.5 \pm 0.03_{m}$	(12) $0.5 \pm 0.02_{m}$	(10) $0.6 \pm 0.05_{n}$
Liver wt (gm)	(10) 20.2 ± 0.91	(12) 22.9 ± 1.27	(10) 20.8 ± 0.94
Liver (% b. wt.)	(10) $3.1 \pm 0.11_{m}$	(12) $3.8 \pm 0.14_{n}$	(10) $4.2 \pm 0.25_{n}$
Spleen wt (gm)	(10) $1.6 \pm 0.18_{x}$	(12) $1.1 \pm 0.12_{x}$	(10) $0.5 \pm 0.08_{y}$
Spleen (% b. wt)	(10) $0.24 \pm 0.02_{x}$	(12) $0.17 \pm 0.02_{xy}$	(10) $0.11 \pm 0.01_{y}$
Testes wt (gm)	(10) $0.3 \pm 0.03_{m}$	(12) $0.4 \pm 0.07_{mn}$	(10) $0.2 \pm 0.02_{n}$
Testes (% b. wt)	(10) $0.04 \pm 0.004_{m}$	(12) $0.06 \pm 0.010_{mn}$	(10) $0.03 \pm 0.003_{n}$

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 14. The effect of feeding rations containing Aroclor 1221, 1254 and 1260 on body, heart, liver, spleen and testes weight of SCWL cockerels.

Parameter	Aroclor	Dietary level (ppm)[a] 0	50	100	150
Body wt. (gm)	1221	(11) 795±57.4[b]	(11) 847±39.4	(10) 904±39.3	(11) 853±23.7
	1254	(11) 840±29.3[c]$_x$	(11) 706±32.5$_y$	(8) 630±34.9$_y$	(7) 635±19.7$_y$
	1260	(11) 786±65.1	(10) 854±43.2	(11) 845±30.9	(12) 787±21.5
Heart wt. (gm)	1221	(10) 5.5±0.69	(11) 4.8±0.23	(11) 4.7±0.28	(10) 4.3±0.31
	1254	(11) 4.7±0.29$_m$	(11) 3.2±0.16$_n$	(8) 3.2±0.19$_n$	(7) 3.4±0.38$_n$
	1260	(11) 4.2±0.39	(10) 4.1±0.26	(11) 4.5±0.29	(12) 3.6±0.23
Liver wt. (gm)	1221	(10) 25.2±1.42	(11) 27.1±0.97	(10) 27.4±1.30	(10) 26.4±1.27
	1254	(11) 25.6±1.49	(12) 24.7±1.52	(8) 26.1±1.70	(7) 30.0±3.20
	1260	(11) 23.4±1.87$_m$	(10) 26.9±1.23$_{mn}$	(11) 29.9±1.37$_n$	(12) 31.0±1.13$_n$
Spleen wt. (gm)	1221	(10) 1.8±0.21	(11) 2.1±0.20	(10) 1.8±0.16	(10) 1.6±0.11
	1254	(11) 1.9±0.11$_x$	(12) 1.3±0.21$_y$	(8) 0.8±0.09$_y$	(7) 0.8±0.08$_y$
	1260	(11) 1.7±0.19	(10) 1.5±0.10	(11) 1.7±0.13	(12) 1.3±0.11
Testes wt. (gm)	1221	(10) 0.9±0.35	(11) 0.8±0.26	(10) 1.0±0.29	(10) 0.4±0.05
	1254	(11) 0.5±0.11	(12) 0.6±0.18	(8) 0.3±0.05	(7) 0.2±0.02
	1260	(11) 1.0±0.36	(10) 1.1±0.51	(11) 1.7±0.50	(12) 0.4±0.07

[a] Rations fed up to 9 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 15. The effect of feeding rations containing Aroclor 1221, 1254 and 1260 on heart, liver, spleen and testes expressed as percentage of body weight of SCWL cockerels.

Parameter	Aroclor	Dietary level (ppm)[a] 0	50	100	150
Heart	1221	(10) 0.6±0.06[b]	(11) 0.6±0.03	(11) 0.6±0.03	(10) 0.5±0.03
	1254	(11) 0.6±0.04	(11) 0.5±0.02	(8) 0.5±0.03	(7) 0.5±0.06
	1260	(11) 0.6±0.05	(10) 0.5±0.02	(11) 0.5±0.03	(12) 0.5±0.02
Liver	1221	(10) 3.0±0.12	(11) 3.2±0.09	(10) 3.0±0.11	(10) 3.1±0.10
	1254	(11) 3.1±0.14[c]$_{m}$	(11) 3.4±0.13$_{m}$	(8) 4.1±0.18$_{n}$	(7) 4.7±0.42$_{n}$
	1260	(11) 3.0±0.11$_{m}$	(10) 3.2±0.09$_{mn}$	(11) 3.6±0.19$_{n}$	(12) 4.0±0.14$_{n}$
Spleen	1221	(10) 0.21±0.010	(11) 0.24±0.017	(10) 0.20±0.010	(10) 0.19±0.010
	1254	(11) 0.23±0.010$_{m}$	(11) 0.18±0.030$_{mn}$	(8) 0.12±0.010$_{o}$	(7) 0.13±0.010$_{no}$
	1260	(11) 0.21±0.014	(10) 0.26±0.010	(11) 0.20±0.010	(12) 0.17±0.010
Testes	1221	(10) 0.10±0.036	(10) 0.10±0.030	(10) 0.11±0.028	(10) 0.05±0.005
	1254	(11) 0.06±0.010	(11) 0.07±0.014	(8) 0.05±0.007	(7) 0.04±0.003
	1260	(11) 0.12±0.041	(10) 0.12±0.053	(11) 0.22±0.070	(12) 0.05±0.008

[a]Rations fed up to 9 weeks

[b]Data are reported as (No. of birds) Mean ± Standard Error

[c]Means having different subscripts are significantly different; m, n ($P<0.05$)

Table 16. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on body, heart, liver, thyroid, testes and comb weights of SCWL cockerels.

Parameter	Dietary Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Body wt (gm)	(15) $746 \pm 65.5^{bc}_{x}$	(29) $583 \pm 54.2_{y}$	(19) $560 \pm 131.4_{y}$
Heart wt (gm)	(15) $4.4 \pm 0.48_{x}$	(29) $3.6 \pm 0.73_{y}$	(19) $4.7 \pm 0.95_{x}$
Heart (% b. wt)	(15) $0.6 \pm 0.06_{x}$	(29) $0.60 \pm 0.11_{x}$	(19) $0.9 \pm 0.19_{y}$
Liver wt (gm)	(15) $16.5 \pm 2.88_{x}$	(29) $12.8 \pm 3.09_{y}$	(19) $18.7 \pm 3.21_{x}$
Liver (% b. wt)	(15) $2.2 \pm 0.36_{x}$	(29) $2.2 \pm 0.48_{x}$	(19) $3.5 \pm 1.11_{y}$
Thyroid wt (mg)	(15) $89.3 \pm 14.40_{x}$	(29) $63.6 \pm 23.65_{y}$	(18) $70.7 \pm 26.81_{xy}$
Testes wt (gm)	(15) $1.3 \pm 1.69_{m}$	(28) $0.4 \pm 0.21_{n}$	(19) $0.4 \pm 0.34_{n}$
Testes (% b. wt)	(15) $0.17 \pm 0.215_{m}$	(28) $0.07 \pm 0.033_{n}$	(19) $0.06 \pm 0.046_{n}$
Comb wt (gm)	(15) $9.4 \pm 3.53_{x}$	(29) $7.2 \pm 4.57_{x}$	(19) $1.32 \pm 1.18_{y}$
Comb (% b. wt)	(15) $1.27 \pm 0.486_{x}$	(29) $1.23 \pm 0.722_{x}$	(19) $0.21 \pm 0.156_{y}$

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean averaged over three replicates ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$) (Scheffe's test)

weight as compared with control values. The most adverse effect on growth expressed as body weight was found among chicks fed the highest levels. No changes in body weight were observed in chickens treated with PCB 1221, 1260 or 1016 at any level fed. The depression in body weight observed in the PCB-treated birds is related to a reduction of feed intake. These observations in early experiments were confirmed with experiment 4. At the end of the eight-week period, the average feed consumption per bird in the group fed the basal ration (ad-libitum) with no PCB was 3.0 kg whereas feed consumption per bird in the treated-group was 1.4 kg. Growth depression has been reported in chickens fed 400 ppm PCB 1242 for three weeks (Flick et al., 1965) and 50-150 ppm PCB 1248 for $4\frac{1}{2}$ to 5 weeks (Rehfeld et al., 1971). The same results were observed by Vos and Koeman (1970) after feeding rats with 2000 ppm Phenochlor DP6 (French PCB) for 60 days.

Mean liver weight and relative liver weight of birds treated with PCB's 1242, 1254 and 1260 showed a significant increase compared with values from the control groups. Similar responses have been observed in rats (Treon, 1956; Grant et al., 1971; Bitman et al., 1972), in rabbits (Vos and Beems, 1971) and different species of birds. This fact has been reported in cockerels (McCune et al., 1962; Flick et al., 1965; Vos and Koeman, 1970; Platonow and Funnell,

1971; Rehfeld et al., 1971; pheasants (Dahlgren et al., 1972) and pigeons (Bailey and Bunyan, 1972).

The heart weight did not show any significant change with PCB's 1221, 1242 and 1260 at any level fed. However, it was significantly decreased with PCB 1254 with levels as low as 50 ppm. Dahlgren et al. (1972), had also found a decrease in the weight of this organ after giving the same PCB by capsule to pheasants. The relative weight of the heart was significantly higher with PCB 1242 with levels $\geq$ 100 ppm. Since no change in actual organ weight was noticed, the decrease in body weight would account for the increase of the relative heart weight observed.

Mean spleen weights of cockerels fed PCB 1254 and 1242 were significantly reduced at levels as low as 50 ppm. The same organ expressed as percentage of body weight in birds fed the same Aroclors showed a lower value compared with those from the control groups. Splenic atrophy has been observed in chickens (Flick et al., 1965; Vos and Koeman, 1970), pheasants (Dahlgren et al., 1972) and rats (Grant et al., 1971) after receiving various PCB's (42% to 60% chlorination).

A depression of the secondary sexual characteristics (comb size) was a consistent observation in birds fed PCB's 1242 and 1254, though it was not quantitated in early experiments. This decrease in comb size indicated reduced

testosterone secretion and was related with changes in testicular weight. The response in birds fed PCB 1242 at 100 ppm was reflected by a decrease in testicular weight as absolute values and when these organs were expressed as percentage of body weight. Even though the other PCB's failed to produce a significant change in testicular weight, the same trend was observed in most instances. A subsequent study confirmed the early observations that related comb size and testes weight. As shown in Table 16, the weights of testes of the PCB treated birds were not different from the values observed with the pair-fed group, however, they were significantly lower than those from the ad libitum group (control). Comb size was significantly higher in birds from the ad libitum and pair-fed groups compared with those values from the treated one. These results are in agreement with those reported previously by other investigators (Platonow and Funnell, 1971; Rehfeld et al., 1971). Thyroid weight was not affected by PCB 1242 (Table 16). No effects on body and organ weights were observed in cockerels fed Aroclor 1016 at 150 ppm (Table 17).

D. Cardiovascular and Hematological parameters

Shown in Tables 18, 19 and 20 are the results on heart rate and mean blood pressure of cockerels fed PCB's 1016, 1221, 1242, 1254 and 1260 at various levels. A significant

Table 17. The effect of feeding rations[a] containing 0 and 150 ppm Aroclor 1016 on body, heart, liver, spleen, thyroid, testes and comb weights of SCWL cockerels.

Parameter	Control		PCB 1016	
	Weight (gm)	% Body wt	Weight (gm)	% body wt
Body	(14) 788±18.0[b]	-----	(11) 817±28.5	_____
Heart	(14) 4.4±0.1	0.6±0.01	(11) 4.5±0.2	0.60±0.01
Liver	(14)19.3±0.6	2.5±0.07	(11)22.3±1.1	2.70±0.11
Spleen	(14) 2.0±0.2	0.3±0.02	(11) 1.8±0.3	0.20±0.04
Thyroid[c]	(14)78.0±5.5	0.01±0.001	(11)94.6±5.5	0.01±0.001
Testes	(14) 0.9±0.3	0.1±0.03	(11) 1.7±0.4	0.20±0.05
Comb	(14) 8.8±1.0	1.10±0.14	(11) 6.7±0.9	0.80±0.10

[a]Rations fed up to 9 weeks

[b]Data are reported as (No. of birds) Mean ± Standard Error

[c]Thyroid weight expressed in mg

Table 18. The effect of feeding rations containing Aroclor 1242 on heart rate and arterial blood pressure of SCWL cockerels.

Parameter	Length of feeding period (weeks)	Dietary levels (ppm) 0	25	50	100	200
Heart rate (beats/min)	8 (trial 1)	(12) 398±8.8$^{ab}_{m}$	(12) 371±10.9$_{mn}$	(11) 361±10.9$_{n}$	(7) 349.±15.5$_{n}$	---
	8 (trial 2)	(10) 388±8.9$_{x}$	---	(12) 351±12.5$_{xy}$	(10) 328±15.3$_{y}$	---
	4 (trial 2)	(6) 436±13.2$_{x}$	---	---	---	(6) 326±23.1$_{y}$
Mean blood pressure[c] (mm Hg)	8 (trial 1)	(12) 137±4.2	(12) 142±2.7	(11) 142±4.6	(7) 149±7.3	---
	8 (trial 2)	(10) 133±3.7	---	(12) 136±6.9	(10) 128±4.8	---
	4 (trial 2)	(6) 133±7.9	---	---	---	(6) 126±8.6

[a] (No. of birds) Mean ± Standard Error

[b] Means having different subscripts are significantly different; m, n, ($P<0.05$); x, y, ($P<0.01$)

[c] Mean blood pressure: MBP = 3/8 PP + DP

Table 19. The effect of feeding rations containing Aroclor's 1221, 1254 and 1260 on heart rate, mean blood pressure and arterial blood pH on SCWL cockerels.

Parameter	Aroclor	Dietary levels (ppm)[a]			
		0	50	100	150
Heart rate (beats/min)	1221	(11) 380±7.9[b]	(11) 380±7.2	(10) 390±11.8	(11) 390±8.0
	1254	(12) 398±8.3[c]$_x$	(12) 372±14.0$_{xy}$	(8) 343±16.5$_y$	(7) 328±14.0$_y$
	1260	(11) 388±14.9	(12) 380±8.7	(12) 366±9.2	(12) 381±10.3
Mean blood pressure[d] (mm Hg)	1221	(12) 150±4.1	(12) 145±9.9	(12) 149±3.8	(12) 148±8.9
	1254	(12) 148±3.2	(12) 148±4.0	(8) 145±10.0	(7) 125±10.4
	1260	(11) 139±4.1	(11) 147±3.8	(10) 148±5.7	(10) 142±3.8
Arterial blood pH	1221	(11) 7.66±0.011	(11) 7.63±0.013	(10) 7.65±0.015	(11) 7.68±0.009
	1254	(12) 7.65±0.010$_m$	(11) 7.65±0.014$_m$	(8) 7.66±0.023$_m$	(7) 7.59±0.017$_n$
	1260	(12) 7.67±0.015	(12) 7.65±0.011	(10) 7.67±0.022	(12) 7.66±0.011

[a]Rations fed up to 9 weeks

[b](No. of birds) Mean ± Standard Error

[c]Means having different subscripts are significantly different; m, n, ($P<0.05$); x, y, ($P<0.01$)

[d]Mean blood pressure: MBP = 3/8 PP + DP

Table 20. The effect of feeding rations containing 0 and 150 ppm of Aroclor 1016 on heart rate and arterial blood pressure of SCWL cockerels.

Treatment[a]	Parameter	
	Heart rate (beats/min)	Mean blood pressure[b] (mm Hg)
PCB control	(13) 403±4.2[c]	(13) 146±1.9
PCB 1016	(11) 393±8.4	(11) 153±2.6

[a]Rations fed up to 9 weeks

[b]Mean blood pressure: MBP = 3/8 PP + DP

[c]Data are reported as (No. of birds) Mean ± Standard Error

decrease in heart rate was observed when PCB 1242 was present in the diet at 100 or 200 ppm, and when PCB 1254 was added in the diet at 100 or 150 ppm. No effect on heart rate or blood pressure was observed from PCB's 1016, 1221, and 1260 at the levels fed. In early experiments, a decrease in heart rate was observed when PCB 1242 was fed to cockerels. It is known that cholinesterase activity is decreased with lowered pH (Hestrin, 1950), hence in experiment 2 arterial blood pH was measured in an attempt to correlate this parameter with the possible change in heart rate. The results in this study were inconclusive because blood pH was only lowered when the cockerels were fed 150 ppm (PCB 1254) whereas bradycardia was observed also at 100 ppm with no change in arterial blood pH (Table 19). Emerson et al. (1964), postulated that the bradycardia observed in dogs acutely treated with endrin was the result of potentiation and/or increased acetylcholine activity because of lowered arterial blood pH.

The effects of PCB 1242 on HCT, hemoglobin concentration, total erythrocyte concentration, MCV and MCHC are presented in Tables 21 and 22. HCT, hemoglobin concentration and total erythrocyte concentration were significantly reduced by PCB 1242, whereas MCV and MCHC were not significantly altered. Tables 23 and 24 show the effect of PCB's 1221, 1254 and 1260 on the same parameters. PCB 1254

Table 21. The effect of feeding rations containing Aroclor 1242 (trial 1) on the packed cell volume, hemoglobin concentration, total erythrocyte count, mean corpuscular volume and mean corpuscular hemoglobin concentration of SCWL cockerels.

Parameter	Dietary levels (ppm)[a]			
	0	25	50	100
Packed cell volume (%)	(12) 27.4±0.83[bc]$_x$	(12) 25.8±0.59$_{xy}$	(10) 23.3±0.86$_y$	(7) 19.8±0.31$_z$
Hemoglobin conc. (gm/100 ml)	(12) 9.24±0.316$_x$	(12) 8.39±0.180$_y$	(11) 7.59±0.205$_y$	(7) 6.29±0.135$_z$
Total erythrocyte count (millions/ mm^3)	(12) 2.77±0.134$_x$	(11) 2.53±0.121$_x$	(10) 2.56±0.096$_x$	(7) 2.00±0.168$_y$
Mean corpuscular volume (μ^3)	(11) 96.1±2.46	(10) 99.2±3.09	(9) 93.9±3.10	(7) 99.7±2.51
Mean corpuscular hemoglobin conc. (gm/100 ml)	(12) 33.8±0.53	(12) 32.6±0.53	(10) 32.7±0.68	(7) 31.8±0.34

[a] Rations fed up to 8 weeks

[b] (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; x, y, z, ($P<0.01$)

Table 22. The effect of feeding rations containing Aroclor 1242 (trial 2) on the packed cell volume, hemoglobin concentration, total erythrocyte count, mean corpuscular volume and mean corpuscular hemoglobin concentration of SCWL cockerels.

Parameter	Length of feeding period (weeks)	Dietary levels (ppm) 0	50	100	200
Packed cell volume (%)	8 wks	(10) 23.4±0.74$^{ab}_{m}$	(12) 20.8±1.56$_{mn}$	(10) 17.3±1.53$_{n}$	---
	4 wks	(6) 25.1±1.40	---	---	(6) 20.6±2.69
Hemoglobin conc. (gm/100 ml)	8 wks	(10) 8.28±0.351$_{x}$	(12) 7.47±0.354$_{xy}$	(10) 5.86±0.638$_{y}$	---
	4 wks	(6) 7.51±0.270	(12) ---	---	(6) 6.24±1.025
Total erythrocyte count (millions/ mm^3)	8 wks	(10) 2.64±0.089$_{m}$	(12) 2.24±0.170$_{mn}$	(10) 1.91±0.192$_{n}$	---
	4 wks	(6) 2.61±0.053	---	---	(6) 2.15±0.300
Mean corpuscular volume (μ^3)	8 wks	(10) 88.8±1.83	(12) 93.8±1.81	(10) 92.3±2.56	---
	4 wks	(6) 96.0±3.92	---	---	(6) 96.8±2.07
Mean corpuscular hemoglobin conc. (gm/100 ml)	8 wks	(10) 35.2±0.68	(11) 34.3±0.62	(10) 33.8±0.95	---
	4 wks	(6) 30.1±0.95	---	---	(5) 31.2±0.37

[a] (No. of birds) Mean ± Standard Error

[b] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 23. The effect of feeding rations containing Aroclor 1221, 1254 or 1260 on the packed erythrocyte volume, total erythrocyte count and hemoglobin concentration of SCWL cockerels.

Parameter	Aroclor	Dietary levels (ppm)[a] 0	50	100	150
Packed erythrocyte volume (%)	1221	(11) 29.5±0.45[b]	(11) 27.4±1.46	(10) 29.5±0.57	(11) 26.7±0.91
	1254	(12) 29.5±0.44[c]$_x$	(10) 23.3±1.36$_y$	(8) 20.4±2.36$_y$	(7) 18.0±1.86$_y$
	1260	(12) 27.6±0.96	(12) 28.8±0.79	(12) 27.4±0.89	(12) 26.6±0.61
Total erythrocyte count (millions/ mm^3)	1221	(11) 2.25±0.061	(11) 2.04±0.096	(10) 2.22±0.103	(11) 2.12±0.157
	1254	(12) 2.16±0.074$_m$	(10) 1.84±0.119$_n$	(7) 1.77±0.168$_n$	(6) 1.48±0.118$_n$
	1260	(11) 2.08±0.096	(12) 2.16±0.077	(12) 1.96±0.063	(12) 1.96±0.063
Hemoglobin conc. (gm/100 ml)	1221	(11) 9.87±0.156	(11) 9.04±0.565	(10) 9.91±0.256	(11) 9.03±0.293
	1254	(12) 10.04±0.182$_x$	(11) 7.91±0.416$_y$	(8) 6.97±0.906$_y$	(7) 6.10±0.728$_y$
	1260	(11) 9.31±0.404	(12) 9.84±0.239	(12) 9.32±0.255	(12) 9.02±0.240

[a] Rations fed up to 9 weeks

[b] (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 24. The effect of feeding rations containing Aroclor 1221, 1254 or 1260 on mean corpuscular volume, and mean corpuscular hemoglobin concentration of SCWL cockerels.

Parameter	Aroclor	Dietary levels (ppm)[a] 0	50	100	150
Mean corpuscular volume (μ^3)	1221	(11) 131.4±1.97[b]	(11) 133.9±3.42	(11) 131.9±4.72	(12) 134.8±5.21
	1254	(12) 137.8±4.44	(8) 134.1±5.16	(7) 127.7±3.28	(6) 130.8±3.78
	1260	(11) 131.9±4.72	(12) 134.8±5.21	(11) 138.6±3.09	(11) 134.7±2.43
Mean corpuscular hemoglobin conc. (gm/100 ml)	1221	(11) 33.4±0.30	(11) 32.8±0.46	(10) 33.5±0.33	(11) 33.9±0.43
	1254	(12) 34.0±0.39	(9) 33.9±0.40	(8) 33.5±0.96	(7) 33.6±1.10
	1260	(12) 33.7±0.63	(12) 34.3±0.44	(12) 34.2±0.47	(12) 33.9±0.27

[a] Rations fed up to 9 weeks

[b] (No. of birds) Mean ± Standard Error

at levels as low as 50 ppm in the diet caused a significant decrease in HCT, hemoglobin concentration, and total erythrocyte concentration, and PCB's 1221 and 1260 had no effect on these parameters at the dietary levels fed. MCV and MCHC were not significantly altered. None of these parameters were affected by Aroclor 1016 when fed at 150 ppm to cockerels (Table 25). Flick et al. (1965), observed changes in HCT and hemoglobin concentration values in cockerels, as early as one week of age after feeding PCB 1242 at 200 and 400 ppm in the diet. These investigators believed that the changes were transient since recovery was observed at three weeks of age. However, the experiment was terminated at that time and no further observations were made. A similar decrease in HCT values and hemoglobin concentration was observed by Rehfeld et al. (1972a,b), when feeding PCB 1248 at levels as low as 20 ppm and 30 ppm for 4½ weeks. These changes were reversible when the PCB was withdrawn after two and one-half weeks. It was observed, during the course of early experiments, that feed consumption diminished considerably in treated birds. Sturkie (1965) has pointed out that some cardiovascular parameters are affected by a decrease of feed ingestion; therefore, a pair-fed experiment was set up to test this possibility. The results on heart rate and blood pressure are shown in Table 26. Both parameters were significantly decreased in birds receiving PCB

Table 25. The effect of feeding rations containing 0 and 150 ppm of Aroclor 1016 on packed erythrocyte volume, total erythrocyte count and hemoglobin concentration of SCWL cockerels.

Treatment[a]	Parameter		
	Packed erythrocyte volume (%)	Total erythrocyte count (millions/mm^3)	Hemoglobin conc. (gm/100 ml)
PCB control	(14) 29.2±0.6[b]	(14) 2.3±0.06	(14) 8.9±0.29
PCB 1016	(11) 27.4±0.7	(11) 2.2±0.06	(11) 9.3±0.23

[a]Rations fed up to 9 weeks

[b]Data are reported as (No. of birds) Mean ± Standard Error

Table 26. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on heart rate and arterial blood pressure of SCWL cockerels.

Parameter	Dietary Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Heart rate (beats/min)	(15) 399±28.6[bc] x	(28) 412±24.6 x	(16) 345±34.1 y
Mean blood pressure[d] (mm Hg)	(15) 140±10.5 x	(29) 146±14.7 x	(15) 113±33.5 y

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean averaged over three replicates ± Standard Error

[c] Means having different subscripts are significantly different; x, y ($P<0.01$) (Scheffe's test)

[d] Mean blood pressure: MBP = 3/8 PP + DP

1242 at 100 ppm. Pair-fed and ad libitum groups were not significantly different from each other, therefore decreased feed intake did not affect heart rate and blood pressure. Table 27 shows the effect of feeding 100 ppm PCB 1242 to cockerels on cardiac output, heart rate, blood pressure, peripheral resistance and stroke volume. Heart rate and blood pressure were significantly lower than those values from birds receiving ration ad libitum or in pair-fed groups. Cardiac output was not affected in any group; however, PCB-treated birds and pair-fed birds showed some lower cardiac output apparently as a result of less feed intake. Vogel and Sturkie (1963) have reported a decreased cardiac output in chickens following starvation. No changes in total peripheral resistance or stroke volume were observed in this experiment. Even though the apparent difference in blood pressure response observed in PCB-treated birds (pair-fed experiment) compared with that of early studies using PCB's 1242 and 1254, the general tendency of this parameter, is apparently to drop when PCB levels increase. Therefore, it is possible that the levels fed would have a borderline action on producing a detrimental effect on blood pressure. The effect of PCB 1242 on the same hematological parameters measured previously are reported in Table 28 for the pair-fed experiment. The significant decline in HCT, total erythrocyte count and

Table 27. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on cardiac output, heart rate, arterial blood pressure, peripheral resistance and stroke volume of SCWL cockerels.

Parameter	Dietary Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Body wt (gm)	(10) 752±24.7$^{bc}_{m}$	(15) 589±14.6$_{n}$	(11) 545±39.6$_{n}$
Cardiac output (ml/min)	(10) 270±20.0	(15) 245±17.3	(10) 247±20.0
Cardiac output (ml/min/Kg)	(10) 360±20.0	(15) 414±30.0	(10) 462±52.0
Heart rate (beats/min)	(10) 396±6.2$_{x}$	(15) 405±5.6$_{x}$	(11) 341±8.4$_{y}$
Mean blood pressure[d] (mm Hg)	(10) 137±4.2$_{m}$	(15) 134±3.7$_{m}$	(11) 105±6.8$_{n}$
Peripheral resistance (mm Hg/ml/min/Kg)	(10) 0.535±0.0443	(15) 0.604±0.0546	(10) 0.446±0.0528
Stroke volume (ml)	(10) 0.68±0.055	(15) 0.60±0.045	(10) 0.73±0.067

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

[d] Mean blood pressure: MBP = 3/8 PP + DP

Table 28. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on total erythrocyte count, hemoglobin concentration, packed erythrocyte volume, mean corpuscular hemoglobin concentration and mean corpuscular volume of SCWL cockerels.

Parameter	Dietary Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Total erythrocyte count (millions/mm^3)	(15) $2.24 \pm 0.273^{bc}_{x}$	(29) $2.39 \pm 0.322_{x}$	(16) $1.23 \pm 0.555_{y}$
Hemoglobin conc. (gm/100 ml)	(15) $8.8 \pm 0.98_{x}$	(27) $9.5 \pm 1.15_{x}$	(16) $5.4 \pm 2.48_{y}$
Packed erythrocyte volume (%)	(15) $27.9 \pm 2.88_{x}$	(29) $30.1 \pm 3.14_{x}$	(16) $16.8 \pm 7.41_{y}$
Mean corpuscular hemoglobin conc. (gm/100 ml)	(15) 31.7 ± 2.09	(27) 31.7 ± 1.84	(16) 31.8 ± 2.25
Mean corpuscular volume (μ^3)	(14) $128.8 \pm 2.65_{m}$	(27) $130.0 \pm 1.77_{m}$	(12) $138.6 \pm 3.69_{n}$

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean averaged over three replicates ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P < 0.05$); x, y ($P < 0.01$) (Scheffe's test)

hemoglobin concentration was in agreement with those from earlier studies. No significant difference in HCT was found among the birds fed rations ad libitum compared with those from the pair-fed group. This is in agreement with the data of Fox and Harrison (1965) who reported no drop in HCT of Japanese quail as a result of 24 to 48 hours of starvation. Hemoglobin concentration or total RBC count also did not change between these two groups. Mean corpuscular volume in PCB-treated birds was found to be significantly ($P < 0.05$) higher than those from pair-fed or ad libitum groups, whereas in previous experiments this parameter did not show any change. It seems that this fact, even though statistically significant, would not be biologically important. The greater percentage decrease in HCT values in relation to the percentage decline in total RBC count in PCB-treated birds compared with values from the other two groups would account for the difference observed in MCV.

The anemia observed when feeding PCB's to chickens could be due to (a) a decrease in total concentration of erythrocytes, (b) a decrease in erythrocyte size, (c) an increase in plasma volume, (d) a decrease in mean corpuscular hemoglobin concentration, or (e) any combination of the above. An increase in plasma volume is improbable since loss in body weight and edema formation was observed with

PCB 1242 at 50 ppm and PCB 1254 at 100 ppm. Edema formation has been reported by other investigators when feeding various PCB's (McCune et al., 1962; Flick et al., 1965; De Vos and Koeman, 1970; Rehfeld et al., 1971). Since there were no differences in MCV and MCHC, the normochromic normocytic anemia observed was due solely to a decrease in total erythrocyte concentration. Abrahamson and Allen (1973) found hypoplastic bone marrow with a decrease particularly in the erythroid series in monkeys intubated with 35 mg/kg of Aroclor 1248. This change may be explained by a decrease in erythropoiesis which is partially controlled by sex steroids. A marked decrease in comb size, indicating reduced testosterone secretion, was observed in birds fed PCB's 1242 and 1254. This observation is in agreement with other workers (Rehfeld et al., 1971; Platonow and Funnell, 1971). Several investigators have reported that testosterone increases total erythrocyte concentration in chickens (Domm et al., 1943; Domm and Taber, 1946; Tanaka and Rosenberg, 1955) and in Japanese quail (Nirmalan and Robinson, 1972). Also, Fried et al. (1966), Alexanian et al. (1967), and Malgor and Fisher (1970) have suggested that testosterone has an effect on erythropoiesis by stimulating in vitro production of erythropoietin in mammalian species.

PCB may decrease red cell formation through enhanced hepatic catabolism of testosterone (Nowicki and Norman, 1972).

Another possibility is that PCB's may directly affect erythropoiesis or erythropoietin production. This is supported by the work of Bitman et al. (1972), who demonstrated that some PCB's have estrogenic-like activity in rats. It has been suggested that estrogens depress erythropoietin precursor formation.

There was a significant relationship between dietary level of PCB's and degree of hydropericardium (Table 11); however, there was no correlation within individual treatments between the magnitude of the bradycardia and the amount of pericardial fluid.

E. Electrocardiogram

In the normal ECG of man P, Q, R, S and T waves are present, representing depolarization and repolarization of atrial and ventricular muscles. The bird ECG shows D, R, S and T waves in the standard limb leads, but there is no Q wave. P represents the depolarization of the atrial muscles and precedes the contraction of the atrium. R and S waves represent depolarization of the ventricles. The T wave corresponds to repolarization of the ventricular muscles (Sturkie, 1949). Tables 29 and 30 show the effect of PCB's 1242 and 1254 at 100 ppm on the electrocardiogram of SCWL cockerels. Bradycardia was a consistent finding in the treated birds when compared with values from control groups. S waves in lead II and III showed significantly

Table 29. The effect of feeding rations containing 0 and 100 ppm of Aroclors 1242 and 1254 on the electrocardiogram of SCWL cockerels.

Parameter	Treatment[a]		
	Control	Aroclor 1242	Aroclor 1254
Heart rate (beats/min)	(12) 429±5.0$^{bc}_{x}$	(10) 361±9.1$_{y}$	(13) 384±6.2$_{y}$
S wave (Lead II) (millivolts)	(12) 0.228±0.017$_{m}$	(10) 0.183±0.014$_{n}$	(13) 0.147±0.010$_{n}$
S wave (Lead III) (millivolts)	(12) 0.213±0.017$_{x}$	(10) 0.185±0.010$_{xy}$	(13) 0.135±0.014$_{y}$
Electrical axis (degrees)	(12) -93.3±1.01	(10) -88.3±2.26	(13) -94.3±3.41

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; x, y ($P<0.01$); m, n ($P<0.05$)

Table 30. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on the electrocardiogram of SCWL cockerels.

Parameter	Dietary Treatment[a]	
	Pair-fed	Aroclor
Heart rate (beats/min)	(29) 457±29.6[bc]$_x$	(23) 379±41.7$_y$
S wave (Lead II) (millivolts)	(29) 0.285±0.016$_x$	(22) 0.184±0.036$_y$
S wave (Lead III) (millivolts)	(29) 0.271±0.016$_x$	(22) 0.171±0.031$_y$
Electrical axis (degrees)	(29) -92.3±8.43	(23) -92.1±8.22

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean averaged over three replicates ± Standard Error

[c] Means having different subscripts are significantly different; x, y ($P<0.01$)

lower voltage than those from the control groups. In most instances, waves in lead I exhibited low voltage in all groups.

Douglas (1960) reported low voltage in ECG's of cockerels. He suggested the air sacs filled with air and the peculiar morphology of the birds would vary conductivity causing a decrease in voltage. Whether the pericardial fluid present in almost all PCB-treated birds acting in the same manner would be responsible for the decrease in conductivity (smaller waves) observed in their ECG's is not clear from this study.

P and T waves were fused in birds from control groups and in those birds with a rather normal ECG in the treated groups. This fusion of both waves was especially apparent in birds which showed a high heart rate. Three birds out of ten fed PCB 1242 at 100 ppm (Exp. 4) showed abnormal ECG's. One bird presented a complete S-A block, skipping 1 or 2 beats and then the heart resumed its normal beat. In Lead III, 6-8 beats were normal, but the next showed large P and R, and small S waves. A second bird exhibited alternating voltage of S waves in an irregular fashion with prominent T or P waves. A third chicken also had, in Lead II, S waves with different voltage. After the short S, prominent R and P waves followed, whereas after the large S wave, a large P was present but the R wave was small or

absent. Lead III had the same characteristics and in addition it showed an incomplete S-A block. No abnormal ECG's were observed in birds treated with PCB 1254 except that waves showed small amplitude compared with those from control birds. Only one PCB-treated bird in Exp. 5 presented abnormal ECG. The record showed a very prominent R and a small S at irregular intervals and an inverted T followed by 6-11 normal beats. Carter and Drury (1929), Sturkie (1950) and Sturkie et al. (1954), have reported abnormal ECG's because of potassium ion and vitamin dificiencies in birds; however, no reports have appeared in the literature in relation to the effect of PCB's on the ECG of birds.

Pathological lesions in the S-A node may cause S-A block; however, post-mortem examination revealed no apparent damage in the hearts of PCB-treated chickens; therefore, the blocks observed may result from a functional rather than a pathological condition.

Values for RS II and RS III were used for electrical axis determination. The results are shown in Tables 29 and 30. This parameter showed no significant change with PCB's 1242 and 1254 at the level fed. This fact would indicate that hydropericardium present in almost all PCB-treated birds would not have any influence on the position of the heart.

F. Sodium and Potassium Analysis

The effects of feeding rations containing 0 and 100 ppm of PCB 1242 on Na^+ and K^+ concentration from plasma of SCWL cockerels are shown in Table 31. K^+ concentration was 20% higher ($P < 0.05$) in PCB-treated birds than in control groups. Sodium concentration in plasma was unchanged at the PCB level fed. On the other hand, Na^+ and K^+ concentrations in pericardial fluid were significantly higher, as compared to these cation levels in plasma of PCB-treated birds (Table 32). The exact mechanism responsible for the increase in the plasma potassium cannot be determined from this study. The influence of some adrenocorticotrophic hormonal mechanism and/or disturbances in renal activity may account for the potassium increase.

G. Thyroxine (T_4) Concentration

The effects of feeding rations containing 0 and 100 ppm of PCB's 1242, on T_4 concentration from serum of SCWL cockerels are shown in Table 33. No significant changes in T_4 concentration were observed in these birds. On the other hand, the same parameter was significantly increased by PCB's 1254 and 1260 at 150 ppm in cockerels, whereas PCB 1221 did not alter T_4 concentration at any level fed (Table 34). Thyroid activity estimated by thyroxine secretion, apparently was enhanced by PCB's 1254 and 1260. Another possibility for explaining the T_4 increase in the serum would be a

Table 31. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on Na^+ and K^+ concentration from plasma of SCWL cockerels.

Parameter	Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Na^+ concentration (m Eq/L)	(11) 145.6±1.20[b]	(17) 139.5±2.40	(16) 139.8±2.03
K^+ concentration (m Eq/L)	(12) $3.5\pm0.17_m$[c]	(17) $3.9\pm0.14_{mn}$	(15) $4.2\pm0.16_n$

[a] Rations fed up to 8 weeks

[b] Data reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$)

Table 32. The effect of feeding rations[a] containing 100 ppm of Aroclor 1242 on Na^+ and K^+ concentration from plasma and pericardial fluid of SCWL cockerels.

Parameter	Plasma	Pericardial Fluid
Na^+ concentration (m Eq/L)	(16) $139.8 \pm 2.03^{bc}_{m}$	(15) $147.8 \pm 2.64_{n}$
K^+ concentration (m Eq/L)	(15) $4.2 \pm 0.16_{x}$	(15) $6.2 \pm 0.51_{y}$

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; m, n ($P<0.05$); x, y ($P<0.01$)

Table 33. The effect of feeding rations containing 0 and 100 ppm of Aroclor 1242 on total serum thyroxine of SCWL cockerels.

Parameter	Dietary Treatment[a]		
	Ad libitum	Pair-fed	Aroclor
Thyroxine µg/100 ml serum	(12) 1.61±0.079[b]	(24) 1.28±0.112	(16) 1.52±0.180

[a] Rations fed up to 8 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

Table 34. The effect of feeding rations containing Aroclor 1221, 1254 or 1260 on total serum thyroxine of SCWL cockerels.

Aroclor	Thyroxine (µg/100 ml serum)		
	Dietary levels (ppm)[a]		
	0	100	150
1221	(5) 1.542±0.19[b]	(5) 2.132±0.19	(5) 1.924±0.24
1254	(5) 1.542±0.19[c] $_{x}$	(5) 1.896±0.09 $_{xy}$	(5) 2.344±0.16 $_{y}$
1260	(5) 1.542±0.19 $_{x}$	(5) 2.094±0.13 $_{xy}$	(5) 2.472±0.15 $_{y}$

[a] Rations fed up to 9 weeks

[b] Data are reported as (No. of birds) Mean ± Standard Error

[c] Means having different subscripts are significantly different; x, y ($P<0.01$)

decreased peripheral utilization of thyroxine by the PCB-treated birds. The exact mechanism responsible for the increase in thyroxine is not clear from this study.

H. Histological Studies

1. Thyroid:--Thyroids from all survivors (PCB-treated birds) in experiment 4 were subjected to histological evaluation and were compared with those (5 birds each) from the pair-fed and ad libitum groups, selected at random. Thyroids from the ad libitum group exhibited follicles with a predominantly cuboidal epithelium filled with colloid. The same gland from the pair-fed groups showed follicles, not quite as large as in the previous groups, lined with cuboidal cells. The histological picture of thyroids of PCB-treated birds was rather variable from bird to bird though most of them showed as a common characteristic a varying degree of lymphoid cell infiltration in the connective tissue; therefore, reducing the number of follicles in the gland. In these cases the follicles surrounded with lymphoid tissue appeared to be more compressed diminishing the colloid content, but the rest of the thyroid presented a fairly normal appearance. It is possible that PCB's may induce some sort of immune response in those birds with lymphocyte infiltration.

2. Testes:--Histological examination of testicular tissue from PCB-treated birds revealed an atrophic state of

the seminiferous tubules in most instances with few undifferentiated germinal cells or no evidence of spermatogenesis. In addition, few Leydig cells were present in the interstitial tissue. Two out of 17 testes studied showed some areas infiltrated with lymphocytes in the connective tissue. Moreover, 2/3 of the PCB-treated birds presented varying degree of eosinophils in the interstitial tissue. In pair-fed and ad libitum groups (5 birds each group picked at random), the seminiferous tubules were lined with germ cells at different stages of differentiation, this process of differentiation being more evident in the latter group. The inhibition of testicular development accompanied by decrease in comb size in White Leghorn cockerels after PCB administration has been reported by Platonow and Funnell (1971). Rehfeld et al. (1971), also observed a decrease in comb size in cockerels after oral ingestion of PCB 1248 for five weeks.

GENERAL DISCUSSION

General cardiovascular and hematological effects, as well as some toxicological aspects of chronic and acute poisoning by some chlorinated hydrocarbons (DDT, endrin and PCB's) were under investigation using SCWL cockerels and adult female chickens.

Previous studies by other investigators have disclosed that toxicity from polychlorinated hydrocarbons varied primarily with a) route of administration, b) level given to the animal, and c) length of time that the compound was administered. Other factors such as nutritional status of the organism, restriction of feed intake, ability to excrete the compound and general health of the bird may influence the biological changes observed in response to a given substance. From a practical standpoint, it is reasonable to question whether the effects observed in these studies are similar to those that occur in the field. Studies in the laboratory permit control of the environment, whereas the situation is completely different in the wild. On the other hand, the presence of other chemicals which might be similarly accumulated by the different animals may enhance additively or synergistically the activity of the compound

being studied. Therefore, considerable caution and reservation must be used in extrapolating from controlled studies to the animal in the field.

The toxicological symptoms observed with DDT and endrin in the present experiments were, in general, in agreement with those reported in the literature by other investigators. Changes in body and organ weights observed after chronic oral administration of these compounds would be an indication of toxicity. Especially important is the increase in liver weight, since this alteration has been related with stimulation of drug metabolism by induction of drug-metabolizing enzymes.

The general toxicological effects, such as tremors, excitability and wing-beat convulsions, observed in acute dosage experiments after endrin administration, would indicate a direct action on the central nervous system. In addition, salivation and cardiovascular alterations (bradycardia and hypertension) would suggest a stimulation on the parasympathetic and sympathetic nervous systems.

Toxicity symptoms after oral administration of PCB's were similar to those described by other workers. One of the most evident symptoms was loss in body weight; this being more noticeable at the end of the experimental period and resulting from the severe decrease in feed intake. Changes in organ weights were a consistent observation.

Liver, heart and spleen weights were altered in most cases. In addition, testes weight was decreased with consequent decrease in comb size. The PCB-treated birds showed testes with small seminiferous tubules with no evidence of spermatogenesis. Risebrough et al. (1968), have reported that pigeons injected intramuscularly with PCB's showed an increase in estradiol metabolites in the liver. The fact that PCB's may induce degradation of steroid hormones by hepatic enzymes; the possibility that some PCB's may have estrogenic-like activity as has been shown in rats (Bitman et al., 1972) or inhibition of pituitary gonadotrophin secretion in the presence of PCB's would account for the depression of secondary sexual characteristics observed in cockerels.

Probably the most consistent finding as a result of PCB toxicity was the presence of fluid in the pericardial sac and abdominal cavity. In one experiment using PCB 1242, 100% of the birds showed varying degrees of hydropericardium. The presence of PCB's apparently influences tissue permeability.

Of all PCB's used in this study, only 1016 did not produce the toxicological symptoms previously described, though 3 out of 15 birds presented a mild degree of hydropericardium.

The cardiovascular alterations observed in cockerels after PCB-treatment were a pronounced bradycardia (PCB 1242

and 1254) or bradycardia and decrease in mean arterial blood pressure (PCB 1242). Parasympathetic nerves supply the heart of birds and that, when stimulated, they tend to slow the heart rate. Therefore, it is possible that PCB's may stimulate the parasympathetic system increasing vagal tone, and in turn producing bradycardia. Another possibility is that the decrease in heart rate observed in birds treated with PCB 1254 was the result of potentiation and/or increased acetylcholine activity because of lowered arterial blood pH. However, the results were inconclusive, as discussed previously, therefore more experimentation would be necessary to properly correlate these two parameters. In addition, the fact that serum K^+ concentration increased in birds given PCB 1242 would contribute to slowing the heart rate. Excess potassium ions in the extracellular fluid are able to weaken the heart and slow down the heart rate (Guyton, 1965). Guyton indicated that these alterations are probably produced by decreasing the resting membrane potential which in turn would decrease the action potential which determines to a great extent the strength of contraction. The exact mechanism responsible for the bradycardia observed in the PCB-treated birds cannot be explained from this study.

The hematological changes observed after chronic oral ingestion of endrin, DDT and PCB, even though in opposite direction, indicate that these compounds would be acting

either directly by stimulating or depressing erythropoiesis, or indirectly by altering the normal production of erythropoietin.

These studies have shown that PCB's 1242 and 1254 are capable of producing important alterations in the hematology and cardiovascular physiology of cockerels. The fact that PCB 1260, 1221 and 1016 produced less toxic effects on these same parameters would indicate one or a combination of the following: 1) that PCB's are not absorbed to the same extent, with the possibility that no absorption may occur for those showing no adverse biological effects; 2) that chickens are able to metabolize these materials to less toxic forms and/or 3) that they excrete these compounds so rapidly that it takes longer for their toxic effects to become apparent.

SUMMARY AND CONCLUSIONS

1. Endrin and DDT did not alter heart rate and mean arterial blood pressure after chronic oral administration to SCWL adult females at any level fed.

2. Significant decreases in HCT values and hemaglobin concentration were observed in adult females when DDT was incorporated into the diet. In contrast, endrin caused a significant increase in these two parameters. Total concentration of erythrocytes showed the same trend as HCT values and hemoglobin concentration, decreasing with DDT and increasing with endrin, therefore these two compounds would be acting, in general, inhibiting or stimulating erythropoiesis. Neither DDT nor endrin produced any change on arterial blood pH at any level fed.

3. In acute experiments, endrin infusion (8-10 mg/Kg) produced a marked bradycardia and hypertension. Since the bradycardia observed occurred after five minutes or simultaneously with hypertension, this fact would indicate no relationship of these two parameters.

4. From the fact that these cardiovascular changes were accompanied with convulsions and excessive salivation, a stimulatory action of endrin on the parasympathetic and sympathetic nervous systems occurred.

5. No significant differences in HCT, total erythrocyte count and hemoglobin concentration were observed, before and after endrin infusion in SCWL adult females.

6. The chronic effect of various dietary PCB's on cardiovascular physiology and hematology of SCWL cockerels was investigated. Heart rate was significantly reduced by PCB's 1242 and 1254 at $\geq$ 100 ppm and no alteration was found with PCB 1221, 1260 or 1016 at 150 ppm. Mean blood pressure was significantly decreased with PCB 1242 at 100 ppm (pair-fed experiment). Even though this parameter was not significantly different in early studies using PCB 1242 or 1254, the general tendency of blood pressure is to drop when PCB levels increase. No detrimental effect on blood pressure was observed with PCB's 1221, 1260 or 1016 at any level fed.

7. Abnormal ECG's were observed in cockerels after chronic PCB ingestion. PCB's 1242 and 1254 produced S waves with lower voltage in leads II and III when they were compared with those from control groups. Also, birds fed PCB 1242 showed S-A block, variable amplitude of S wave in an irregular fashion with prominent T or P waves and inverted T waves. Electrical axis, determined by the RS complex, showed no significant change with PCB's 1242 or 1254 at the level fed.

8. PCB 1242 fed at a rate of 100 ppm did not produce any significant effect on cardiac output, stroke volume and peripheral resistance of SCWL cockerels.

9. Hemoglobin concentration, HCT and total erythrocyte count were found to be significantly decreased by PCB's 1242 and 1254 at $\geq$ 50 ppm. These same parameters were not affected by PCB's 1221, 1260 and 1016 at any level used. It was concluded that the anemia observed was due solely to a decrease in total erythrocyte concentration. The possibility that these changes may be due to a decrease in erythropoiesis was discussed.

10. K^+ concentration was significantly higher in cockerels fed PCB 1242 at 100 ppm whereas, no change in Na^+ concentration was observed in these birds. On the other hand, both Na^+ and K^+ concentration were significantly higher, when they were compared with plasma levels in the PCB-treated birds.

11. Toxicological symptoms observed in cockerels fed PCB's included 1) depressed body weight, 2) general edema and hydropericardium, 3) increase in liver weight and decrease in heart, spleen and testes weight, 4) depression of the secondary sexual characteristics (decrease in comb size) and 5) a varying degree of mortality. Also, histological observations showed thyroids of PCB-treated birds to have lymphoid cell infiltration in the connective tissue in an

irregular fashion, whereas, the rest of the gland presented a fairly normal appearance. Histological examination of testicular tissue revealed an atrophic state of seminiferous tubules with few undifferentiated germinal cells, no evidence of spermatogenesis, few Leydig cells and presence of eosinophils in the interstitial tissue.

12. There was a significant relationship between the dietary level of PCB's and the degree of hydropericardium; however, there was no correlation within individual treatments between the magnitude of the bradycardia and the amount of pericardial fluid.

13. Based on the different parameters analyzed in this study the toxicity of the various PCB's used is summarized as follows: PCB 1242 > PCB 1254 > PCB 1260 > PCB 1221 > PCB 1016.

APPENDIX

APPENDIX

Aldrin - (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethanonaphthalene)

Benzene hexachloride (lindane) - 1,2,3,4,5,6-hexachlorocyclohexane

Chlordane - 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan

DDD - 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane

DDE - 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene

DDT - 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane

Dieldrin - (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene)

Endrin - (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene)

Heptachlor - 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

Isodrin - 1,2,3,4,10,10-hexachloro-1,4,5,8-diendomethylene-1,4,4a,5,8,8a-hexahydronaphthalene

Methoxychlor - 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane

Telodrin - 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-endomethylenenaphthalene

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