THE EFFECTS OF POLYBROMINATED BIPHENYLS ON THE CARDIOVASCULAR PHYSIOLOGY OF THE SINGLE COMB WHITE LEGHORN COCKEREL

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ABSTRACT

THE EFFECTS OF POLYBROMINATED BIPHENYLS
ON THE CARDIOVASCULAR PHYSIOLOGY
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By

Frederick William Heineman

FireMaster FF-1, a commercial grade hexabromobiphenyl (PBB) used in the thermoplastics industry, was inadvertently mixed into dairy cattle rations by a major supplier of livestock feed in the State of Michigan. The toxic effects of PBB's are similar to those of polychlorinated biphenyls (PCB) and chlorinated insecticides, both of which affect the cardiovascular system. This study was undertaken to determine whether the chronic ingestion of PBB's could affect cardiovascular performance.

Two hundred and five three-day-old Single Comb White Leghorn cockerels were divided into five equal groups and fed rations containing 0, 75 or 150 ppm of FireMaster FF-1. In studies by other investigators PBB's caused a decrease in feed consumption. To separate any effects of the restriction of feed intake from those of the PBB's, a control group (0 ppm) was pair-fed with each level of treatment.

After 8 weeks of feeding the cockerels the experimental diets, statistically significant differences ($p = 0.05$) were

found between treatment groups and their paired controls. Cardiac output and cardiac index decreased, heart rate decreased, hematocrit and hemoglobin decreased, total peripheral resistance increased, frontal plane mean electrical axis exhibited left axis deviation, electrocardiogram wave amplitude decreased and body weight decreased. There appeared to be a direct relationship between the concentration of PBB's in the diets and the degree of change. No significant differences were found in blood pressure (mean arterial and pulse pressures), stroke volume and respiratory rate. The general symptoms of PBB intoxication observed among PBB-fed birds were retarded comb growth, ascites, reduced feed consumption and increased mortality. The results of this study indicated that the chronic ingestion of FireMaster FF-1 can have a depressive affect on the performance of the cardiovascular system.

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INTRODUCTION

Production and use of halogenated hydrocarbon compounds have expanded greatly since the early 1940's. This group of chemicals includes the chlorinated insecticides, polychlorinated biphenyls (PCB's) and, more recently, polybrominated biphenyls (PBB's). These compounds are similar in structure, and share many of the same chemical and biological properties. The halogenated hydrocarbons tend to be very stable, almost insoluble in water, and highly soluble in fats and oils. They tend to concentrate in the lipid-rich tissues of animals exposed to very low environmental concentrations. Daphnia magna, for example, have been shown to contain polychlorinated biphenyl (PCB) concentrations 48,000 times that of their environment (Sanders and Chandler, 1972).

Polybrominated biphenyls were first produced in commercial quantities in 1970. They were used to increase the fire resistance of thermoplastics. During 1973, several hundred pounds of commercial grade hexabromobiphenyl (FireMaster FF-1) was mistaken for a feed ingredient and incorporated into livestock feed (Dunckel, 1975). As a result, dairy and beef cattle, poultry, swine, sheep and, indirectly, humans were contaminated throughout the state of Michigan. PBB's were not implicated as the contaminant until April of 1974.

The majority of research efforts concerning exposure to PBB's dealt with their excretion, absorption, tissue distribution, alterations of reproduction, and hepatic effects. Since polychlorinated biphenyls and organochloride insecticides can alter heart rate and other cardiovascular parameters, it was thought that polybrominated biphenyls might have a similar effect. The purpose of this study was to determine whether chronic exposure to commercial grade hexabromobiphenyl could influence the performance of the cardiovascular system.

LITERATURE REVIEW

I. Properties of FireMaster FF-1

FireMaster FF-1 is comprised of biphenyls of varying degrees of bromination. Hexabromobiphenyl isomers are the primary constituents of the product, with the 2,2',4,4',5,5'-hexabromobiphenyl isomer accounting for the largest portion of these (Jacobs et al., 1976). At least three other hexabromobiphenyl isomers are found in FireMaster. Tetra-, penta-, and heptabromobiphenyl homologues make up the rest of the product, along with a few biphenyls of higher degrees of bromination. The average number of bromine atoms per molecule is six, giving FireMaster a bromine content of approximately 75 per cent.

Commercial preparations of PCB's have been shown to contain highly toxic chlorinated naphthalenes and dibenzofurans (Vos et al., 1970; and Bowes et al., 1973). O'Keefe (1976), using high resolution mass spectroscopy, determined that naphthalene contaminants also occur in commercial hexabromobiphenyl. Peaks were seen representing approximately 25 ppm hexabromonaphthalene and 1 ppm pentabromonaphthalene. Evidence was also found that brominated benzenes were present in FireMaster, however, no proof of tetra-, penta-, or hexabromodibenzofurans was noted.

Polybrominated biphenyls are a solid at 26°C with a specific gravity of 2.57. The product's vapor pressure is low, as is the solubility in water (11 ppb). PBB's are highly soluble in most organic solvents, lipids, and oils, with an octanol:water partition coefficient of 240,000. Decomposition occurs at temperatures above 300°C (Kerst, 1974, Norris et al., 1975). Octabromobiphenyls have been shown to undergo dehalogenation and ring methylation more readily than PCB's (Ruzo and Zabik, 1974). Jacobs et al., (1976) found PBB's to be very stable in soils with only minor incorporation occurring into plants. Leaching of PBB's into ground water was also minimal (Filonow, Jacobs and Mortland, 1976).

II. Absorption, Tissue Residues, and Excretion of Polybrominated Biphenyls

The ease with which halogenated hydrocarbons cross biological membranes appears to be inversely related to the molecular weight (Bailey and Bunyan, 1972). Consequently, the more highly brominated biphenyls and, hence, those of greater molecular weight, tend to be absorbed and metabolized to a lesser extent (Fries et al., 1973; Sosa-Lucero, Dela Inglesia and Thoma, 1973; and Villeneve and Khera, 1975).

Absorption of polybrominated biphenyls can occur via three routes: the respiratory mucosa, the ectoderm (conjunctiva and epidermis), and the gastrointestinal tract. Octabromobiphenyl (OBB), at a concentration of 2.6 mg/liter of inspired air, has been shown to produce liver enlargement in rats (Aftosmis et al., 1972b). In the same study, contact exposure

of the eyes of rabbits to OBB caused only transient conjunctival irritation. Dermal application resulted in no irritation to intact skin and only minor erythema and edema to abraded skin. The dermal dose of hexa- and octabromobiphenyls necessary to elicit liver changes in rabbits was 1 g/kg of body weight. Most toxicity studies of polybrominated biphenyls (PBB) used feeding as the mode of administration. The results of these investigations are discussed in the following sections.

FireMaster FF-1 was accidentally substituted for Nutri-Master (MgO) in dairy cattle feed at a feed mixing plant in Michigan (Dunckel, 1975). Cross contamination of foods for other species occurred through the use of common mixing, shipping, and storage facilities, and the use of contaminated animal products in other feeds. Fries et al., (1975) and Detering et al., (1975a) determined the tissue residues in dairy cows 9 to 12 months after exposure. PBB concentrations ranged from 108 to 2480 ppm in body fat and 3.0 to 3.5 ppm in blood. Human exposure occurred among persons who consumed products of contaminated animals. Blood, milk, and adipose tissue samples from these people contained PBB residues of up to 2.26, 92.66, and 174 ppm, respectively (Dunckel, 1975). The ratio of PBB concentrations among the different tissues depends upon the amount of PBB consumed, the length of time over which exposure occurred and the time elapsed since exposure (Lee et al., 1975; Gutenmann and Lisk, 1975; Babish, Gutenmann and Stoewsand, 1975; and Fries et al., 1975). Aftosmis et al., (1972b), Matthews and Anderson (1975) and Norris et al., (1975) found that PCB's and PBB's were quickly removed from the blood and

stored in the liver and muscle. Redistribution occurred with time and the compounds and their metabolites accumulated in adipose tissue (Babish et al., 1975 and Lee et al.,1975). This process resulted from the high lipid:water partition coefficient for PBB's which also resulted in slow diffusion out of fat. In the case of exposure to high dietary levels of PBB's (1,000 ppm), the adipose concentration in rats returned to normal feed continued to increase for several weeks, while muscle and liver PBB content declined (Lee et al., 1975).

Polybrominated biphenyls are excreted in the feces and lipid containing products. Norris et al., (1975) intubated rats with radio-labelled octabromobiphenyls (OBB) and found that less than one per cent of the specific activity of the original dose was eliminated in the urine and expired air. Fecal specific activity, which accounted for the remaining excretion, declined rapidly during the initial 24 hours after dosing, then declined slowly for the duration of the experiment. Norris et al., (1975) proposed that OBB elimination from rats was described by a diaphasic curve. The first component of the curve characterized a biological half life of less than one day. The second component defined a half life of greater than 16 days. Detering et al., (1975b) found the biological half life of PBB's in Holsteins to be 84 days.

The rate of PBB excretion differed between male and female Japanese quail (Fries et al., 1973 and Babish et al., 1975). Egg producing female quail had an increased capacity

to eliminate the contaminants. This effect was attributed to the additional loss of PBB's to the lipid portion of the eggs. In chickens, 58% of the dietary intake of PBB's was excreted in eggs (Ringer and Polin, 1976). The milk fat in lactating mammals can, in the same manner, augment fecal PBB excretion. In contaminated cows, milk fat PBB concentrations were up to 600 times those of the blood (Detering et al., 1975b).

The ease with which halogenated hydrocarbons cross biological membranes is inversely proportional to the molecular weight. As a result, the biological half lives of the different PBB homologues are not the same. Fries et al., (1973) reported heptabromobiphenyls to be excreted more rapidly than hexabromobiphenyls, presumably because the heptabromobiphenyls were not absorbed from the gut or deposited in adipose as readily as the hexabromobiphenyls. The heptabromobiphenyl homologue was concentrated in the feces relative to hexabromobiphenyls.

III. Effects of Polybrominated Biphenyl Intoxication

Polybrominated biphenyls incorporated into diets decreased the feed consumption of rats (Farber and Baker, 1974), cows (Jackson and Halbert, 1974; and Prewitt, Cook and Fries, 1975) and chickens (Lillie et al., 1975; Cecil and Bitman, 1975; and Kowaleski, 1976). Increased mortality and decreased growth occurred in PBB-fed chickens (Cecil and Bitman, 1975).

Norris et al., (1975) reported decreased hematocrits and erythrocyte counts in rats fed octabromobiphenyls. These findings are consistent with those for PCB-fed primates (Allen,

Carstens and Barsotti, 1974) and cockerels (Iturri, 1974). It has not been determined whether the anemia associated with halogenated hydrocarbon toxicity is due to shortened red cell life or alterations of erythropoiesis.

Accumulation of extravascular fluid, particularly in the pericardial and peritoneal cavities, was a consistent finding in chickens fed PCB's (McCune, Savage and O'Dell, 1962; Flick, O'Dell and Childs, 1965; and Iturri, 1974). Rats (Norris et al., 1975) and cockerels (Kowaleski, 1976) fed PBB's also displayed general body edema. A possible explanation for halogenated hydrocarbon-produced edema was reported by Bayer and Bird (1974). These investigators noted that chicks fed PCB's exhibited fenestration and proliferation of the epicardial lining, which could result in the loss of fluids and osmotically active substances to the pericardial space.

Hepatic hypertrophy occurred in laboratory animals fed PBB's (Farber and Baker, 1974; Norris et al., 1975; Babish et al., 1975; and Kowaleski, 1976). The pathologic changes seen in liver cells from rats exposed to PBB's included proliferation of the smooth endoplasmic reticulum, depletion of particulate glycogen, peripheral displacement of the rough endoplasmic reticulum, increased numbers of lipid inclusions, and central lobular cell necrosis (Lee et al., 1975).

Microsomal enzyme activity in rat (Farber and Baker, 1974) and Japanese quail (Cecil, Harris and Bitman, 1975) livers was induced by polybrominated biphenyls to a greater extent than by DDT or PCB's. The increase in hepatic enzyme

activity found by Cecil et al., (1975) increased pentobarbital metabolism. Studies with DDT and PCB's demonstrated that the rate of hormone metabolism is also accelerated by hepatic microsomal enzyme induction. Animals treated with halogenated hydrocarbons had lower plasma concentrations and increased clearance of androgens (Conney, 1967; Risebrough et al., 1968; Bradlow et al., 1973; and Freeman and Idler, 1975), thyroxine (Bastomsky, 1974 and Bastomsky and Murthy, 1976), and glucocorticoids (Brown et al., 1957; Balezs and Kupfer, 1966; Conney, 1967; Wassermann and Wassermann, 1973 and Sanders and Kirkpatrick, 1975) due to induction of liver enzymes. Aminolevulinic acid synthetase (ALAS) activity was stimulated in Japanese quail fed PBB's or PCB's (Strik, 1973a and Strik, 1973b). The enhanced ALAS activity combined with induced activity of other liver enzymes produced porphyria in these birds.

Polychlorinated biphenyls fed to rats decreased blood thyroxine (T_4) concentration by stimulating the hepatic T_4 degradation and displacing T_4 from the serum binding proteins (Bastomsky, 1974). Biliary T_4 excretion was increased by a factor of five, accounting for the goitrogenic effect seen in animals exposed to PCB's. Since PBB's are several times more potent than PCB's as hepatic microsomal enzyme inducers (Farber and Baker, 1974 and Cecil et al., 1975), they would presumably stimulate and even greater increase in hepatic metabolism of thyroxine. In agreement with this, Kowaleski (1976) found cockerels fed PBB's developed enlarged thyroid glands.

Plasma glucocorticoid concentrations may also be decreased by hepatic microsomal enzyme induction (Conney, 1967). Brown et al., (1957) suggested that the action of DDD (See Appendix A) in decreasing blood glucocorticoid concentrations and in causing adrenocortical atrophy was attributable to a direct effect on the adrenal gland. The goitrogenic effects of halogenated hydrocarbons would also be expected to cause increased thyroid stimulating hormone (TSH) release. Increased TSH release under the influence of goitrogens is accompanied by a decrease in blood ACTH concentration, and results in adrenocortical atrophy (Nocenti, 1968).

Avian and mammalian reproductive capacities appear to be adversely affected by exposure to polybrominated biphenyls. Lillie et al., (1975) and Cecil and Bitman (1975) reported a decrease in the hatchability of eggs from PBB-fed hens. In mammals, embryotoxicity resulting from maternal ingestion of PBB's was described by Detering et al., (1975b) and Prewitt et al., (1975). Embryonic exposure occurred by placental PBB transfer in mammals (Detering et al., 1975a) and by the contaminated egg yolk in aves (Fries et al., 1975; and Cecil and Bitman, 1975). Fetal weight (Corbett, Beaudoin and Cornell, 1975) and progeny growth (Lillie et al., 1975) decreased as a result of maternal contamination. Cecil and Bitman (1975) did not find any difference in fertility between PBB-fed and control hens.

Secondary sexual characteristics of cockerels fed PBB's were retarded in development (Kowaleski, 1976). Similar

observations of anti-androgenic activity have been reported for birds fed chlorinated hydrocarbons (Platonow and Funnell, 1971 and Iturri, 1974).

IV. Cardiovascular Effects of Chlorinated Hydrocarbons

Acute Effects

Endrin, (See Appendix A) an insecticide, caused bradycardia and systemic hypertension when infused intravenously into dogs (Emerson, Brake and Hinshaw, 1964) and White Leghorn hens (Iturri, 1974). Reins et al., (1966) reported that the increased blood pressure in dogs after acute exposure to Endrin was due to an increase in cardiac output ($\dot{Q}$) with no concurrent change in total peripheral resistance. $\dot{Q}$ increased in response to augmented venous return as a result of vasoconstriction of the splanchnic and hepatic circulations. Increased catecholamine secretion may have caused the redistribution of blood volume (Dinman, 1974). Hinshaw et al., (1966) observed increased blood catecholamine concentrations in adrenalectomized, Endrin-treated dogs. The increased secretion of catecholamines was attributed to enhanced activity of sympathetic nerves. Hinshaw and his co-workers also found an increase in $\dot{Q}$, but not in blood pressure, after Endrin administration. In the same investigation, Endrin caused increased left atrial pressures in isolated dog hearts, apparently by a direct depressive action on the ventricles.

DDT infusion sensitized the myocardia of cats, dogs, rabbits and monkeys to exogenous catecholamines. Doses of

epinephrine which evoked transient hypertension followed by bradycardia in control animals caused ventricular fibrillation in treated animals. When the centrally mediated convulsions resulting from exposure to high concentrations of DDT were blocked with curare, epinephrine still caused fibrillation. The sensitization of the myocardium to catecholamines was concluded to be independent of the anoxia produced by convulsions (Phillips, Gilman and Crescitelli, 1946). Lethal oral doses of Aldrin (See Appendix A) in cats (Gowdey et al., 1952) and DDT in rats (Henderson and Woolley, 1970) also sensitized the heart to catecholamines.

Many of the alterations of cardiovascular performance during acute intoxication by chlorinated insecticides are elicited through effects on the central nervous system (Phillips and Gilman, 1946). Nerve cell membrane permeability to sodium ions and the time course of increased transmembrane sodium ion flux increased when exposed to chlorinated hydrocarbons (Åhrem et al., 1974). The neurons were thus in a state of partial depolarization and, hence, hyperexcitable.

Bilateral vagotomy (Gowdey et al., 1954) and atropine (Gowdey et al., 1952; Emerson et al., 1964; and Iturri, 1974) eliminated insecticide-produced bradycardia. Reins et al., (1966) suggested that chlorinated hydrocarbons evoke an increase in vagal activity. Concurrently, increased catecholamine concentrations produced vasoconstriction and hypertension (Reins, Holmes and Hinshaw, 1964).

Acidosis occurred in dogs after the infusion of Endrin (Emerson et al., 1964). Acidosis causes decreased acetylcholinesterase activity (Guyton, 1976). In agreement with this, Gowdey et al., (1952) found that Aldrin prolonged and potentiated the actions of acetylcholine. When Emerson et al., (1964) prevented a change in blood pH by buffering, bradycardia did not occur.

Chronic Effects

Ingestion of up to 35 mg of DDT per day for one year did not alter heart rate or blood pressure in humans (Hayes, Dale and Pirkle, 1971). Similar studies did not produce an effect on heart rate in turkeys (Simpson, Thompson and Nelson, 1972). Homing pigeons fed low doses of DDT (3 mg/kg body weight/day) developed tachycardia, but higher doses caused bradycardia (Jefferies and French, 1971b). In the same study, both levels of doses to bengalese finches evoked tachycardia only. Iturri (1974) reported decreased heart rates in cockerels fed Aroclor 1242[a] or Aroclor 1254[a]. Aroclor 1242 also caused hypertension, but no change was observed in cardiac output. Dogs receiving 150 to 300 mg of DDT per kg of body weight had increased cardiac outputs (McNamara, Bing and Hopkins, 1946). Hypotension and bradycardia were found in dogs treated with DDT (Reins et al., 1964). Danopoulos, Melissinos and Katsas, (1953) found evidence of myocardial degeneration in humans accidently contaminated

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[a]Aroclor is Monsanto Corporation's tradename for polychlorinated biphenyls.

with hexachlorocyclohexane. Decreased thickness and tone of the ventricular walls were observed in cockerels fed PCB's (Iturri, 1974) and PBB's (Kowaleski, 1976). Iturri (1974) also reported that PCB's caused hyperkalemia and acidosis.

Injections of epinephrine and norepinephrine evoked smaller increases in blood pressure and myocardial contractility in dogs fed DDD than in normal dogs (Osorio and Kraemer, 1958; and Cueto, 1970). Prior to catecholamine administration no differences existed in heart rate and blood pressure between treated and control animals. After the transient epinephrine-induced hypertension and tachycardia subsided, the DDD-treated dogs developed hypotension and bradycardia.

Electrocardiogram (ECG) S wave amplitude was decreased in PCB-fed cockerels (Iturri, 1974). A few of these birds also exhibited inverted T waves and S-A blocks. Jefferies and French (1971a) found an increase in ECG amplitude in pigeons fed 3 mg of DDT per kg of body weight and a decrease at higher levels. Variable T wave voltage and increased QRS intervals occurred in heifers treated with Aldrin (Rumsey and Bond, 1972). Danopoulos et al., (1953) found that hexachlorocyclohexane decreased the S-T segment of ECG in humans. Cardiac arrythmias developed in DDT-poisoned rabbits (Judah, 1949).

The bradycardia observed in animals chronically exposed to chlorinated hydrocarbons may have resulted from increased vagal tone and/or altered acetylcholinesterase activity (Reins et al., 1964, and Iturri, 1974). Jefferies and French (1971a)

proposed that the dichotomous response in heart rate and ECG amplitude for high and low doses of DDT was caused by altered blood thyroxine concentrations. DDT stimulated thyroxine secretion at low levels, but at higher levels acted as a goitrogen. The diminished positive inotropic response to catecholamines seen in DDD-fed dogs was attributed to the effects of decreased circulating glucocorticoids (Osorio and Kraemer, 1958 and Cueto, 1970).

OBJECTIVES

1. To determine the effects of feeding rations containing FireMaster FF-1 (PBB) on the cardiac output, heart rate, stroke volume, blood pressure, total peripheral resistance, electrocardiogram, hematocrit and hemoglobin concentration of Single Comb White Leghorn (SCWL) cockerels.

2. To evaluate the possible relationship between hydropericardium in PBB-fed SCWL cockerels and the performance of the cardiovascular system.

3. To determine whether the possible changes of the cardiovascular parameters in PBB-fed cockerels resulted from the restriction of feed intake caused by PBB's.

MATERIALS AND METHODS

FireMaster FF-1[a,b] was pulverized in a mortar and pestle and passed through a sieve (U. S. Standard, number 30) to obtain a small enough particle size to assure uniform distribution of the PBB's in the diets. Thirty grams of the ground PBB's were mixed with 2970 g of sieved basal ration.[c,d] Aliquots of this 1% PBB premix were combined with the basal ration (Table 1) to yield diets containing 75 to 150 ppm PBB. Mixing was done on a Paul G. Abbé, Inc., feed mixer (Little Falls, N. J.) by tumbling for 15 minutes in 30 lb. capacity feed cans. Analysis of the diets mixed by this method were found to be within 2.5% of error of the calculated concentrations of PBB's.

In order to compensate for the decrease in feed consumption associated with chronic PBB ingestion, a control group

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[a]Obtained from Michigan Chemical Company, Chicago, Illinois.

[b]FF-1 is the form of FireMaster which was accidentally mixed into cattle feed. The FF-1 used in this project was from the same lot of FireMaster which was involved in the contamination of livestock in Michigan.

[c]Dietary components are listed in Table 1.

[d]All rations were finely ground to assure uniform texture of the diets.

Table 1. Basal ration

Ingredients	Grams/Kg.
Corn	618.5
Soybean meal, 49%	202.0
Alfalfa meal, 17%	25.0
Meat and bone meal, 50%	30.0
Fish meal, 60%	30.0
Dried whey	20.0
Oats	50.0
Salt	2.5
Ground limestone	7.5
Dicalcium phosphate	7.5
Vitamin and mineral premix	5.0
Additives	1.0

was pair-fed with each treatment level. The control and treatment groups were fed starting at three days of age in the following manner:

ad libitum control.....	basal ration *ad libitum*
75 ppm treatment.....	basal ration plus 75 mg/kg PBB, *ad libitum*
Pair-fed to 75 ppm, control....	basal ration limited to the 75 ppm treatment group's rate of consumption
150 ppm treatment.....	basal ration plus 150 mg/kg PBB, *ad libitum*
Pair-fed to 150 ppm, control...	basal ration limited to the 150 ppm treatment group's rate of consumption

The rates of feed consumption for the *ad libitum* control, 75 ppm treatment and 150 ppm treatment groups were determined daily.

Each group contained forty-one three-day-old Single Comb White Leghorn (SCWL) cockerels. The chickens were weighed and assigned to groups such that the group means and variances for starting body weights were equal. The animals were housed in standard chick starting batteries. At five weeks of age they were transferred to growing batteries for the duration of the experiment. At five and nine weeks, ten birds from each group were randomly chosen for the determination of hematocrits and hemoglobin concentrations. Blood was withdrawn by venipuncture into heparinized glass capillary tubes, centrifuged for five minutes at 4,500 rpm using an International Micro-

capillary Centrifuge (International Equipment Co., Boston, Massachusetts). Hematocrits were determined on a microcapillary reader. Twenty microliters of blood from each bird was used to determine the hemoglobin concentrations by the cyanmethemoglobin method (Lynch et al., 1969). After eight weeks on the experimental diets fifteen individuals were randomly selected from each group for the determination of cardiac output and blood pressure. At the conclusion of the project, ECG's were recorded and the pericardial fluid volumes measured.

Cardiac outputs were measured using a dye dilution technique (Hamilton et al., 1932). The cockerels were weighed, received a local anesthetic (0.5 to 1.0 ml of 1% procaine HCl, subcutaneously at the site of the incision), and were restrained on a small animal operating board. The left carotid artery was isolated and cannulated in the direction of the heart, proximal to its bifurcation, with PE90 tubing (Intramedic, Clay-Adams, Inc., New York, N. Y.) filled with heparinized physiological saline solution. The cannula connected the artery to a Harvard Infusion/Withdrawal pump (model 950, Harvard Apparatus, Millis, Massachusetts) and a Statham Physiological Pressure Transducer (model P23Ac, Statham Laboratories, Inc., Hato Rey, Puerto Rico) via a series of three-way valves and tubing. Carotid arterial pressures were recorded from the pressure transducer on a Grass model 5A polygraph at a chart speed of 5 mm per second. Blood pressure recordings were made immediately prior to and after the measurement of cardiac output. Mean arterial pressure, pulse pressure, heart rate and

respiratory rate were calculated from the blood pressure recordings.

During the cardiac output determinations, approximately 3 ml blood was withdrawn from the carotid artery through a dye tracer cuvette (Gilson Medical Electronics, Middleton, Wisconsin) by the Harvard pump. The cuvette's diffraction grating had been previously adjusted using arterial and venous blood from a non-experimental bird so that changes in absorbance due to the hemoglobin oxygen content were minimal. The withdrawal pump was equipped with a lubricated 10 ml glass syringe and adjusted to withdraw 10.3 ml per minute. The electrical signal from the dye tracer control unit (model DLT, Gilson Medical Electronics, Middleton, Wisconsin) was amplified by a Grass model 7P1 low-level D.C. pre-amplifier and model 7DA D.C. driver amplifier and ultimately recorded on an Esterline Angus Analog Recorder (12 inch paper width, model E1101E, Esterline Angus Instrument Co., Inc., Indianapolis, Indiana) at a chart speed of 12 inches per minute. The cardiac output apparatus was calibrated by passing *in vitro* blood samples containing 0 or 5 mg of dye per liter through the cuvette. This was done at the beginning and at the end of each day that cardiac outputs were determined.

As blood was drawn from the carotid artery through the cuvette, the baseline was set. Two tenths of a milliliter per kilogram of body weight of Cardio-Green (0.5 mg/ml; Hynson, Westcott and Dunning, Inc., Baltimore, Maryland) was injected rapidly into the left jugular vein. When recirculation of the

dye was evident from the dilution curve, the pump was reversed and the blood reinfused. After recording the post-cardiac output blood pressure, the carotid artery was ligated, the incision closed and the cockerel returned to its group.

To correct for dye recirculation, the initial portion of the descending limb of the dye dilution curve was extrapolated logarithmically using the least squares regression line to within one per cent of baseline (Appendix B). The area under the resulting curve was estimated with a compensating polar planimeter (model 4236, Keuffel and Esser Co., Hoboken, New Jersey) and used to calculate the cardiac output by the following formula:

$$\text{Cardiac Output} = \frac{\text{mg of dye injected}}{\text{area under the dilution curve in mg min/L}}$$

Cardiac output, diastolic and systolic pressures, and heart rate were used in the following calculations: (See Appendix B)

$$\text{Cardiac Index} = \frac{\text{Cardiac output in ml/min}}{(\text{Body weight in kg})^{0.734}}$$
(Speckmann and Ringer, 1963)

$$\text{Stroke Volume} = \frac{\text{Cardiac output in ml/min}}{\text{Heart Rate in beats/min}}$$

Mean Arterial Pressure = Diastolic pressure + 3/8 pulse pressure in mm of Hg
(Sturkie, 1976)

$$\text{Total Peripheral Resistance} = \frac{\text{Mean arterial pressure}}{\text{Cardiac output in L/min}}$$

ECG's were recorded from leads I, II, and III on a Grass model 5A polygraph at a chart speed of 50 mm per second with

the cockerels restrained in a normal posture. The algebraic sum of R and S wave voltages from leads II and III were used to plot the mean electrical axes.[a] The birds were killed by cervical dislocation. Pericardial fluid volume was measured by opening the thoracic cavity and inserting a 26 gauge needle into the pericardial space. Any fluid present was withdrawn into a 1, 3, or 10 ml syringe, depending on the volume.

All data were analyzed, with the exception of pericardial fluid volumes, by one-way analysis of variance. Multiple comparisons among group means were made using the Student-Neuman-Kuel's test (Sokal and Rohlf, 1969). The group variances for pericardial fluid volumes were significantly different, hence, these group means could not be compared by ANOVA. They were analyzed using the non-parametric Wilcoxon Rank-Sum test (Mendenhall, 1971). Correlation coefficients (r) were tested for significance for ECG wave amplitude versus pericardial fluid volume, cardiac output versus pericardial fluid volume and cardiac output versus heart rate.

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[a]Unlike humans, lead I in aves is normally isoelectric (Sturkie, 1976).

RESULTS

Average daily feed consumption per bird for the PBB-fed groups and the ad libitum control group is plotted in Figure 1. The 75 ppm treatment group consumed 7.5% less feed during the experiment than the ad libitum control group. The pair-fed control groups consumed all of their feed, hence, the feed intake for the pair-fed groups was the same as for the PBB-fed groups. The average total amount of polybrominated biphenyls ingested during the study was 237 mg/kg and 510 mg/kg of body weight for the 75 ppm and the 150 ppm treatment groups, respectively. The highest rate of mortality (27.5%) occurred in the 150 ppm treatment group (Figure 2). Mortality in the group pair-fed to 150 ppm was 22.5%. In the ad libitum control and the 75 ppm pair-fed control groups mortality was 7.5%. The lowest mortality (2.5%) occurred in the 75 ppm treatment group.

Body weights at eight weeks of age were less for the PBB-fed birds than for the ad libitum control birds (Table 2). While no statistical difference existed between mean body weight of the 75 ppm treatment group and that of its pair-fed control, the mean body weight of the 150 ppm treatment group was less than that of its pair-fed control. The decrease in body weight with respect to the ad libitum control group, resulting from the restriction of feed intake in the pair-fed

Figure 1. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on feed consumption of SCWL cockerels.

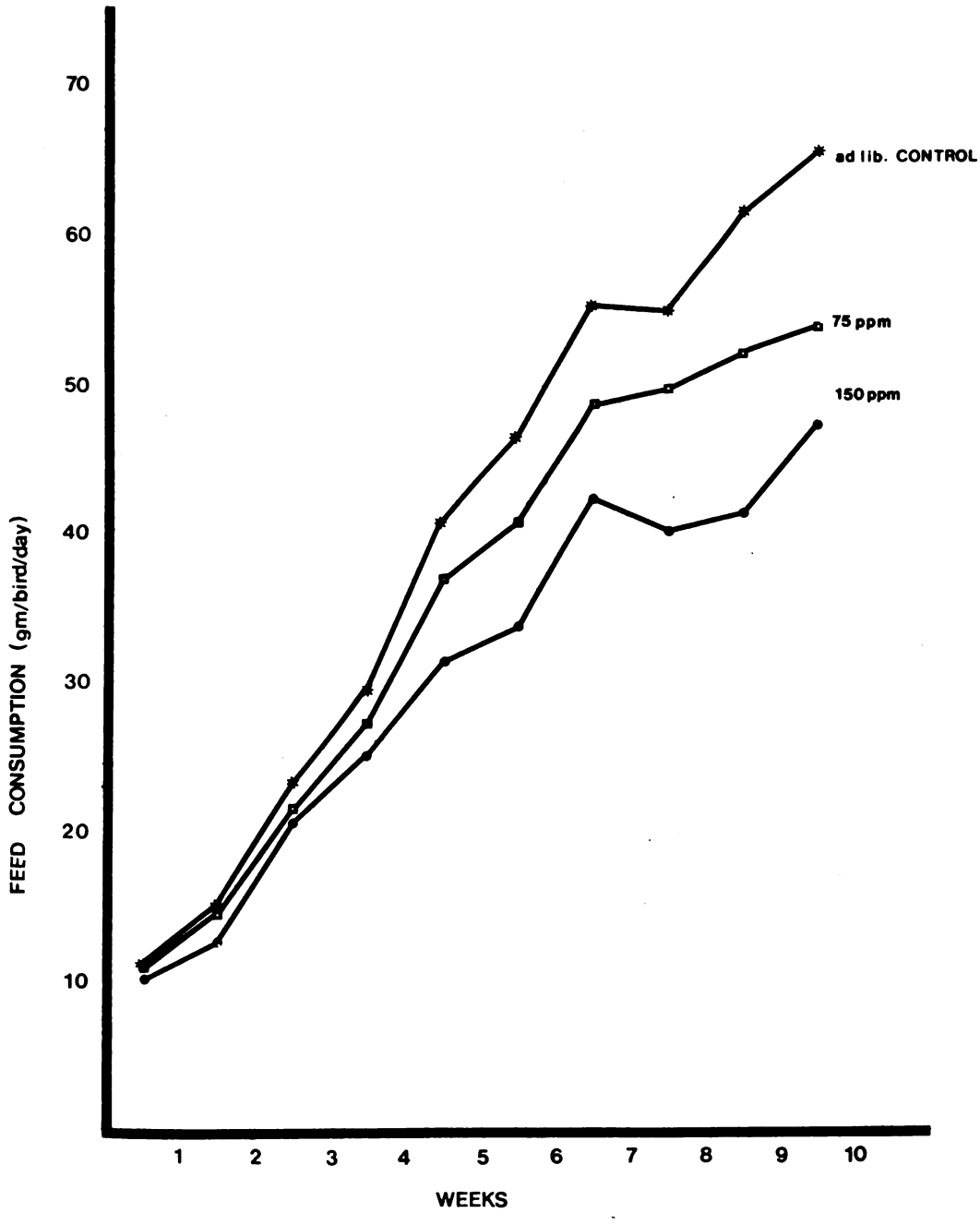

70
60
50
40
30
20
10
FEED CONSUMPTION (gm/bird/day)
ad lib. CONTROL
75 ppm
150 ppm
1
2
3
4
5
6
7
8
9
10
WEEKS

Figure 2. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on mortality of SCWL cockerels. X = weeks receiving experimental diets. Y = total % mortality.

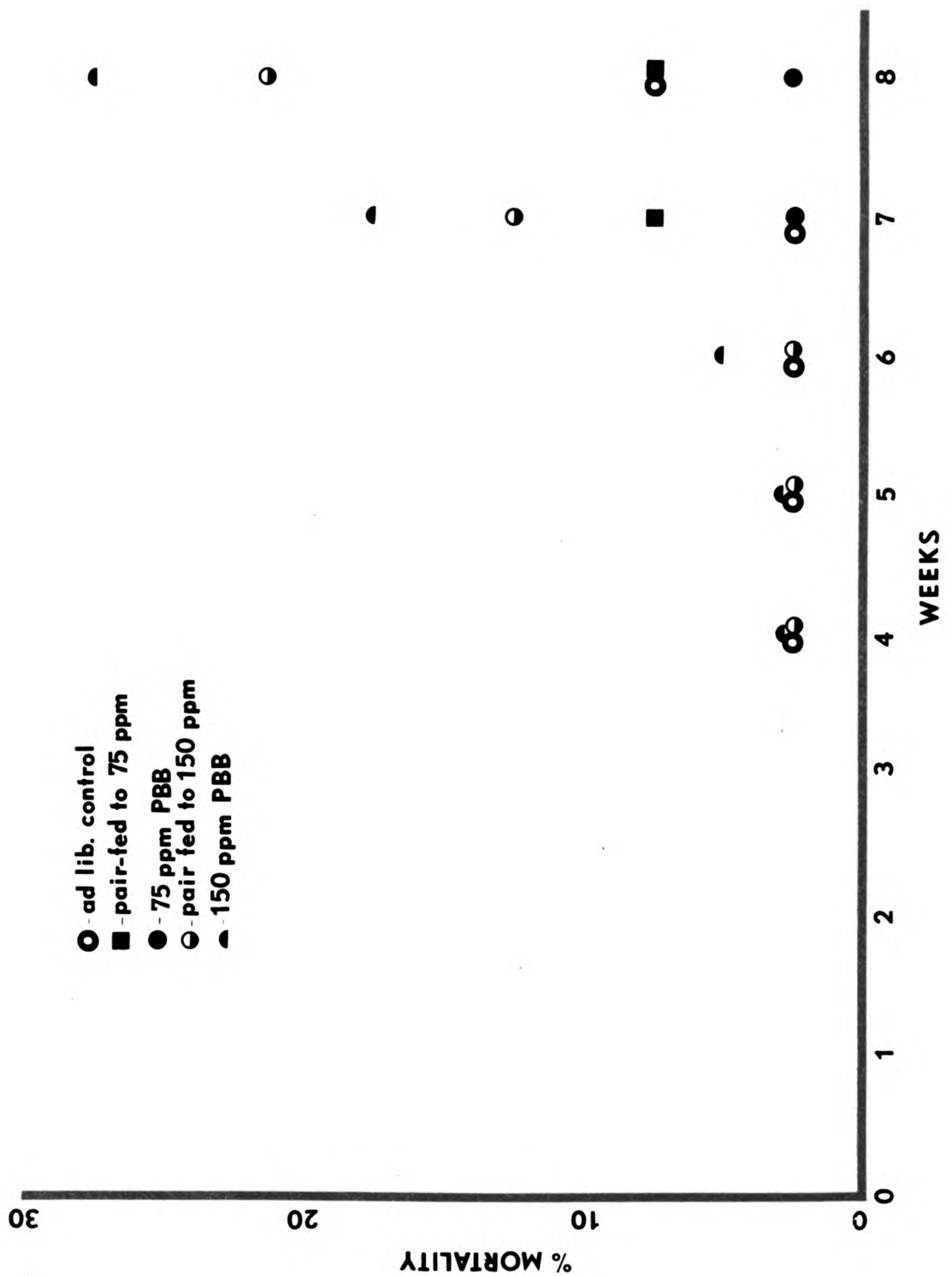

ad lib. control
pair-fed to 75 ppm
75 ppm PBB
pair fed to 150 ppm
150 ppm PBB
% MORTALITY
0
10
20
30
0
1
2
3
4
5
6
7
8
WEEKS

Table 2. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on body weight and pericardial fluid volume of SCWL cockerels.

Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
n=	15	15	15	15	15
Body weight (gm)	$774 \pm 17_{w}$	$733 \pm 18_{w,x}$	$712 \pm 19_{x}$	$627 \pm 14_{y}$	$573 \pm 21_{z}^{bc}$
n=	12	13	14	16	14
Pericardial fluid volume (ml)	$0.20 \pm 0.08_{x}$	$0.12 \pm 0.03_{x}$	$0.96 \pm 0.26_{x}$	$0.22 \pm 0.06_{x}$	$3.86 \pm 1.02_{y}$

[a]Rations fed for 8 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

control groups, was significant only for the group pair-fed to 150 ppm.

Pericardial fluid volume was increased in the group receiving rations containing 150 ppm PBB (Table 2.) No statistically significant differences were found among the ad libitum control, 75 ppm PBB, pair-fed to 75 ppm and pair-fed to 150 ppm groups.

After 5 weeks of feeding the experimental diets, the group fed the diet containing 150 ppm of PBB had a significantly lower mean hematocrit than its paired control group (Table 3). At the end of the experiment (9 weeks), both the 75 ppm and 150 ppm PBB treatment groups exhibited hematocrits significantly lower than their respective pair-fed controls. Similarly, the hemoglobin concentration of the PBB-fed groups were significantly decreased at 9 weeks of age.

Bradycardia was found in cockerels fed the 150 ppm PBB treatment diet (Table 4). The difference between the 150 ppm treatment and 150 ppm pair-fed group means represents an 18% decrease in heart rate. No significant changes occurred in pulse pressure or respiratory rate. No significant differences for mean arterial pressures were found between treatments and pair-fed controls at either dietary PBB concentration, however, the mean arterial pressure of the 150 ppm PBB group was significantly less than that of the ad libitum control group.

Cardiac output and cardiac index of PBB-fed cockerels were decreased relative to the pair-fed controls (Table 5). The cardiac index of the pair-fed control groups was also

Table 3. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on hematocrit and hemoglobin concentration of SCWL cockerels (10 birds/group).

Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
Hematocrit: (%)					
5 weeks	$29.6 \pm 0.8_{x}$	$30.2 \pm 1.2_{x}$	$27.7 \pm 0.8_{x,y}$	$30.0 \pm 0.8_{x}$	$25.9 \pm 0.6_{y}^{bc}$
9 weeks	$34.6 \pm 0.3_{x}$	$35.3 \pm 0.7_{x}$	$28.1 \pm 0.9_{y}$	$33.8 \pm 0.4_{x}$	$23.1 \pm 1.0_{z}$
Hemoglobin (gm/dl)	$9.9 \pm 0.1_{x}$	$10.2 \pm 0.2_{x}$	$8.3 \pm 0.3_{y}$	$10.0 \pm 0.2_{x}$	$6.7 \pm 0.3_{z}$

[a]Rations fed for 9 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

Table 4. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on heart rate, respiratory rate, mean arterial pressure and pulse pressure of SCWL cockerels (15 birds/group).

Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
Heart rate (beats/min)	$381 \pm 10_x$	$383 \pm 7_x$	$362 \pm 15_x$	$376 \pm 10_x$	$308 \pm 13_y^{bc}$
Respiratory rate (cycles/min)	$35 \pm 1_x$	$37 \pm 2_x$	$37 \pm 2_x$	$42 \pm 2x$	$38 \pm 3_x$
Mean arterial pressure (mm Hg)	$150 \pm 4_x$	$153 \pm 3_x$	$144 \pm 3_{x,y}$	$142 \pm 4_{x,y}$	$135 \pm 5_y$
Pulse pressure (mm Hg)	$31 \pm 2_x$	$32 \pm 2_x$	$32 \pm 2_x$	$29 \pm 1_x$	$26 \pm 2_x$

[a]Rations fed for 8 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

Table 5. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on cardiac output, cardiac index, stroke volume and total peripheral resistance of SCWL cockerels (15 birds/group).

Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
Cardiac output (ml/min)	$232 \pm 12_{x,y}$	$282 \pm 19_{x}$	$224 \pm 11_{y}$	$247 \pm 16_{x}$	$182 \pm 17_{y}^{bc}$
Cardiac index ($ml/min/kg^{0.734}$)	$281 \pm 15_{x}$	$356 \pm 25_{y}$	$288 \pm 12_{x}$	$347 \pm 20_{y}$	$276 \pm 25_{x}$
Stroke volume (ml)	$0.612 \pm 0.035_{x}$	$0.739 \pm 0.052_{x}$	$0.643 \pm 0.051_{x}$	$0.658 \pm 0.038_{x}$	$0.586 \pm 0.042_{x}$
Total peripheral resistance (PRU)	$674 \pm 42_{x}$	$574 \pm 38_{x}$	$663 \pm 34_{x}$	$608 \pm 40_{x}$	$829 \pm 73_{y}$

[a]Rations fed for 8 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

significantly greater than that of the ad libitum control. For the 150 ppm PBB treatment group, total peripheral resistance (TPR) increased with respect to all other groups. Stroke volume did not differ significantly among any of the groups.

The effect of PBB feeding on the electrocardiograms (ECG's) is presented in Tables 6 and 7. Mean electrical axis shifted significantly in a positive direction for the cockerels receiving rations containing 150 ppm PBB. Lead II S wave voltage (Table 6) decreased significantly for both PBB-fed groups when compared to those of controls. Lead II R wave voltage was not altered, but RS complex voltage was decreased in 150 ppm PBB-fed birds with respect to the 150 ppm pair-fed control group. In lead III (Table 7) no significant differences were found between the treatment groups and their pair-fed controls for R wave, S wave and RS complex voltages. Lead III S wave voltage for the ad libitum control group was significantly greater than those of either of the treatment groups. Lead III RS complex voltage for the ad libitum control group was significantly greater than those of the treatment groups and the pair-fed control groups. Figure 3 gives an example of an ECG (lead II) from the 150 ppm PBB treatment group illustrating the typical changes found, namely, low S wave amplitude, bradycardia, and near-isoelectric RS complex. No other consistant abnormalities were observed in the ECG's of PBB-treated animals.

Significant correlations were found to exist at $p=0.05$ for heart rate versus cardiac output and for pericardial fluid

Table 6. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on mean electrical axis, lead II R and S wave voltages and lead II RS complex voltage of SCWL cockerels (10 birds/group).

Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
Mean electrical axis (degrees)	$-89 \pm 6_x$	$-75 \pm 11_x$	$-68 \pm 12_x$	$-88 \pm 6_x$	$+31 \pm 18_y^{bc}$
Lead II: (mv)					
R wave voltage	$+0.066 \pm 0.011_x$	$+0.123 \pm 0.024_x$	$+0.081 \pm 0.014_x$	$+0.106 \pm 0.017_x$	$+0.137 \pm 0.023_x$
S wave voltage	$-0.335 \pm 0.036_x$	$-0.272 \pm 0.026_x$	$-0.196 \pm 0.020_y$	$-0.224 \pm 0.053_x$	$-0.168 \pm 0.019_y$
RS complex voltage	$-0.269 \pm 0.044_x$	$-0.149 \pm 0.045_y$	$-0.115 \pm 0.029_{y,z}$	$-0.163 \pm 0.024_y$	$-0.031 \pm 0.024_z$

[a]Rations fed for 9 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

Table 7. The effect of feeding rations containing 0, 75 and 150 ppm of FireMaster FF-1 (PBB) on lead III R and S wave voltages and lead III RS complex voltage of SCWL cockerels (10 birds/group).

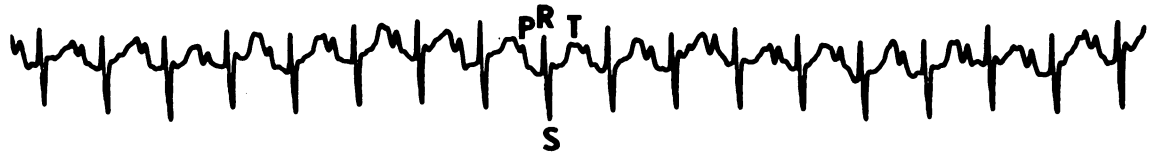
Parameter	Dietary Treatment[a]				
	Ad libitum control	Pair-fed to 75 ppm	75 ppm PBB	Pair-fed to 150 ppm	150 ppm PBB
Lead III: (mv)					
R wave voltage	+0.027 $\pm 0.004_x$	+0.056 $\pm 0.016_x$	+0.029 $\pm 0.004_x$	+0.056 $\pm 0.014_x$	+0.080[bc] $\pm 0.023_x$
S wave voltage	-0.282 $\pm 0.029_x$	-0.228 ± 0.021 x,y	-0.178 $\pm 0.007_y$	-0.217 $\pm 0.013_{x.y}$	-0.172 $\pm 0.016_y$
RS complex voltage	-0.254 $\pm 0.030_x$	-0.172 $\pm 0.029_y$	-0.149 $\pm 0.025_y$	-0.162 $\pm 0.017_y$	-0.092 $\pm 0.024_y$

[a]Rations fed for 9 weeks

[b]Data reported as Group mean ± Standard Error

[c]Means having different subscripts are significantly different (P=0.05)

Figure 3. The effect of feeding rations containing 0 and 150 ppm of FireMaster FF-1 (PBB) on the electrocardiogram of SCWL cockerels. Chart speed=50mm/second; 1 mv=2.5 cm; Lead II



Pair-fed to 150 ppm Heart rate=360 beats/min

150 ppm PBB Heart rate=250 beats/min

volume versus lead II S wave voltage. The relationship was not significant pericardial fluid volume and cardiac output.

Although no measurement of comb size was made, the PBB-fed cockerels appeared to have much smaller combs than the control birds. Since comb growth in SCWL chickens is under the influence of androgens, either the circulating concentration or the effect of androgens was modified. Cockerels which were most severely affected by PBB-intake, some of which died prior to completion of the experiment, seemed to have respiratory difficulties (slow and labored breathing), and exhibited muscular weakness, feather erection, and, finally, aphagia. Upon post-mortem examination, these cockerels were found to have severe hydropericardium and ascites. Pair-fed control cockerels which died during the course of the experiment did not exhibit these symptoms.

DISCUSSION

I. Effect of polybrominated biphenyls on feed consumption, mortality and body weight.

In agreement with the findings of Kowaleski (1976), cockerels fed polybrominated biphenyls exhibited a reduction in the rate of consumption proportional to the concentration of PBB's in the diet (Figure 1). The mechanism through which PBB's affect appetite has not been studied. The PBB's might have simply made the rations unpalatable. It is also possible that PBB's could have altered the blood concentrations of hormones which effect the appetite, such as thyroxine (T_4) and glucocorticoids. A third possible mechanism could be via an effect on the hypothalamic satiety and hunger centers. These centers are responsive to alterations of the concentrations of circulating substrates, especially glucose and amino acids. If PBB ingestion in some way altered the concentrations of these substrates in the blood, the activity of the hypothalamic feeding centers would have been affected. Another possible way by which polybrominated biphenyls could have decreased appetites would have been through a direct effect on the hypothalamus.

Mortality rates were comparable between treatment and pair-fed control groups (Figure 2). This observation supports the conclusion of Cecil and Bitman (1975) that the mortality

caused by feeding PBB's was primarily the result of decreased feed intake. The deaths of PBB-fed cockerels and the pair-fed controls in some cases appeared to involve factors other than feed restriction, such as decreased resistance to disease and other stresses. Iturri (1974) reported 43% mortality among Single Comb White Leghorn (SCWL) cockerels fed rations containing 100 ppm Aroclor 1242. In contrast, feeding SCWL cockerels rations containing 150 ppm PBB resulted in 27.5% mortality (Figure 2). Apparently, Aroclor 1242 is more toxic than are polybrominated biphenyls.

Another parameter related to the degree of feed restriction was body weight. The decrease in body weight found among PBB-fed cockerels was directly proportional to the concentration of PBB's in the diets and the degree of restriction of feed intake. The fact that the mean body weight of the 150 ppm treatment group was significantly less than that of its pair-fed control group (Table 2) may indicate that other factors in addition to the restriction of feed intake influenced body weights. Apparently, the efficiency of feed utilization by the cockerels fed rations containing 150 ppm of PBB's was decreased. Strik (1973b) and Cecil et al., (1975) found that PBB's induced hepatic microsomal enzyme activity. Continued hepatic conjugation of compounds, resulting from induced microsomal enzyme activity, can lead to depletion of the endogenous constituents of the conjugative process. In growing animals these endogenous constituents, such as glucuronate and cysteine, may not be available in sufficient quantities to supply both

conjugation and growth when fed a microsomal enzyme inducer (Dinman, 1974). In such animals, growth is retarded. The hepatic microsomal enzymes, when induced, also increased the rate at which the liver metabolizes hormones (Sanders et al., 1974; Bastomsky and Murthy, 1976). Certain hormones metabolized by hepatic pathways, notably thyroxine (T_4) and glucocorticoids, are necessary in normal concentrations for growth (Nocenti, 1973; Murlow, 1973). If the plasma concentrations of T_4 and/or glucocorticoids were decreased by PBB's, it could account for the decreased efficiency of feed utilization seen in the 150 ppm PBB treatment group. The possibility also exists that PBB's may have altered the absorption of nutrients from the gastrointestinal tract. Regardless of the mechanism(s) by which PBB's acted, the net effect at the 150 ppm PBB dietary concentration was to decrease the efficiency of feed utilization.

II. Effect of polybrominated biphenyls on pericardial fluid volume, hematocrit and hemoglobin concentration.

Fluid accumulation in the pericardial and peritoneal cavities can result from increased capillary pressure, increased capillary permeability, decreased plasma colloid osmotic pressure and blockage of the lymphatic drainage, or a combination thereof (Guyton, 1976). The finding of increased pericardial fluid volume among the cockerels fed rations containing 150 ppm PBB (Table 2) was consistent with the findings of other investigators administering PBB's (Kowaleski,

1976) and polychlorinated biphenyls (McCune et al., 1962; Flick et al., 1965; Kuratsune et al., 1972 and Iturri, 1974). Despite the numerous studies documenting the occurrence of hydropericardium in chickens fed halogenated hydrocarbons, only Bayer and Bird (1974) have reported a possible mechanism of transudate accumulation. These investigators observed fenestration and proliferation of the epicardium in cockerels fed PCB's. The resulting loss of osmotically active solute and fluid to the pericardial space may have accounted for the development of hydropericardium. Pericardial fluid may have accumulated by a similar mechanism in PBB-fed birds.

Anemia is defined as a condition in which erythrocyte numbers, size or hemoglobin content is decreased. The cockerels in this study which were fed PBB's were found to be anemic since there was a reduction in hematocrit and a decreased hemoglobin concentration (Table 3). Since no change in hematocrit or hemoglobin was observed in the pair-fed control groups, the anemia was not due to the restriction of feed intake. The mechanism by which anemia occurred in cockerels fed PBB's is uncertain, however, based on the findings of a few related studies, some speculations can be made about the possible cause of the anemia. Strik (1973b) observed porphyria in PBB-fed quail. Porphyric alterations can cause erythrocyte maturation disorders and can adversely effect hemoglobin synthesis and thus produce anemia (Erslev and Gabuzda, 1974). Androgens, glucocorticoids, T_4, and pituitary hormones can influence erythropoiesis (Guyton, 1976). Any effect PBB's might have

had on the circulating levels of these hormones would be expected to alter red blood cell production. PCB's have been shown to increase the rate of hepatic T_4, glucocorticoid, and androgen metabolism (Sanders et al., 1975). Thyroxine transport was also altered by PCB's due to competition between chlorine and iodine for binding sites on T_4-binding proteins (Bastomsky, 1974). Norris et al., (1975) suggested that bromine ions derived from PBB's may have competed with iodine in a similar manner. Decreased thyroxine transport would result in lower concentrations of circulating T_4. Polybrominated biphenyls could have decreased erythrocyte production through a direct effect on the process of erythropoiesis or by decreasing the life expectancy of red blood cells. Although the means by which hematocrit and hemoglobin were decreased in PBB-fed cockerels was not determined, the anemia was clearly not the result of feed restriction since the hematocrits and hemoglobin concentrations of pair-fed controls remained comparable to those of the ad libitum control.

III. Effect of polybrominated biphenyls on cardiovascular parameters.

Normally, heart rate is determined by the time interval required for the spontaneous depolarization of the cardiac pacemaker cells to reach their membrane threshold potential. This interval is shortened by stimuli which increase heart rate, such as epinephrine and increased activity of the cardiac sympathetic nerves, and is lengthened by stimuli which decrease heart rate, such as increased activity of the vagus. Although

bradycardia was statistically significant only in cockerels fed rations containing 150 ppm PBB, there appeared to be a trend towards depression of heart rate in the PBB-fed groups (Table 4). The bradycardia associated with intoxication by chlorinated hydrocarbons has been attributed to increased vagal tone, inhibition of acetylcholinesterase (AchE), hyperkalemia (Reins et al., 1964; Emerson et al., 1964; and Iturri, 1974) and hypothyroidism (Jefferies and French, 1971b). Polybrominated biphenyls might have caused bradycardia by effecting similar mechanisms. Hyperkalemia, however, is associated with characteristic abnormalities in electrocardiograms (Goldman, 1973) which did not appear to occur in the ECG's of cockerels fed PBB's. Kowaleski (1976) reported enlarged thyroid glands in cockerels receiving rations containing PBB's. If the goitrogenic effect of PBB's was due to a decrease in the concentration of circulating thyroxine, a depressive effect on the heart would be expected. According to Guyton (1976) the plasma concentration of T_4 and heart rate are directly related due to thyroxine's influence over the metabolic oxygen requirement and due to a direct, positive inotropic effect of T_4 on the myocardium. Thus, bradycardia is a sympton of hypothyroidism. At this time no studies of the thyroid function, vagal tone or acetylcholinesterase activity in animals fed PBB's have been reported. Further investigation, particularly concerning the endocrine function, central nervous system involvement and AchE activity during PBB intoxication, is needed to define the cause of bradycardia

p. 43 follows p. 61

$\dot{Q}$ among the cockerels of the pair-fed control groups. Iturri (1974) noted no change in $\dot{Q}$ among pair-fed control cockerels. Complete restriction of feed intake has been reported to decrease cardiac output in chickens (Vogel and Sturkie, 1963). This apparent contradiction could not be explained, and further $\dot{Q}$ studies using specific levels of food restriction would be in order.

Total peripheral resistance (TPR) was significantly increased in cockerels fed rations containing 150 ppm PBB (Table 5). Since mean arterial pressures did not change significantly (Table 4) despite the decrease in cardiac outputs of the PBB-fed birds, TPR may have increased as a result of reflex vasoconstriction. If the increased TPR was the result of a reflex attempt to maintain blood pressure homeostasis, the vasoconstriction could have been mediated centrally, by the baroreceptors, or locally, by the Bayliss reflex. Cockerels fed rations containing PCB's exhibited no change in $\dot{Q}$ and hypotension (Iturri, 1974). This indicated that the response of the SCWL cardiovascular system to PBB's and PCB's was apparently dissimilar.

Anemia generally causes increased cardiac output and tachycardia due to increased activity of the cardiac sympathetic nerves and to decreased peripheral resistance. The hypoxia resulting from the decreased oxygen transporting capacity of anemic blood evokes sympathetic discharge to the heart, centrally, and vasodilation, locally. The local vasodilation, and decreased blood viscosity in anemias where

hematocrit decreases, serve to decrease peripheral resistance. The resultant increase in cardiac output maintains 0_2 delivery to the tissues, except in cases of extreme anemia (Guyton, 1976). PBB-fed cockerels exhibited the opposite response (Tables 3, 4, and 5). Despite decreased hematocrit and hemoglobin concentration caused by feeding rations containing PBB's, cardiac output decreased, TPR increased and heart rate decreased relative to those of the pair-fed controls. It is evident that PBB's had a depressive effect on the cardiovascular system in spite of the existence of anemia. Whether the cardiovascular effect of PBB's can be attributed to a direct effect on the myocardium and vasculature or to an alteration of the endocrine and/or central nervous system activities has not yet been determined.

IV. Effect of polybrominated biphenyls on electrocardiogram (ECG)

Changes in ECG's and cardiac electrical axes are often indicative of changes within or surrounding the myocardium. The frontal plane mean electrical axes of chickens normally average between -74.04 and -102.11 degrees (Sturkie, 1976). Certain ventricular conditions can cause abnormal deviations in the axes. These include change in the anatomical orientation of the heart, hypertrophy of one ventricle, bundle branch block and destruction of cardiac muscle (Guyton, 1976). Only the 150 ppm PBB treatment group mean electrical axis differed significantly from that of its pair-fed control (Table 6). The left axis deviation found in the group fed rations containing

150 ppm PBB indicates the occurrence of physical displacement of the myocardium to the left, left bundle branch block, left ventricular hypertrophy or muscular destruction in the right ventricle. Ventricular hypertrophy and abnormal orientations were not observed during the post-mortem examinations of the PBB-fed cockerels. Blockage of the conductive system of the heart and ventricular dilation or hypertrophy increases the time required for ventricular depolarization and, thus, increases the length of the RS complex (Guyton, 1976). The duration of the RS complexes did not appear to be altered in the electrocardiograms (ECG's) of the PBB-fed cockerels (Figure 3), hence, the conductive tissue of the PBB-fed cockerels was presumably normal. Kowaleski (1976) reported that the hearts of cockerels fed PBB's were flaccid, thin-walled and weighed less than those of controls. If myocardial degeneration occurred in the birds fed PBB's, it could account for the left axis deviation and for the decrease in lead II S wave and RS complex voltages (Table 6). Hydropericardium is also a cause of decreased ECG voltage in the standard limb leads (Goldman, 1973). Indeed, the correlation between pericardial fluid volume and lead II S wave voltage in the PBB-fed cockerels was significant ($R= -0.744$). A third possible cause of decreased ECG voltage is pulmonary emphysema (Guyton, 1976). Iturri (1974) found that the ECG's of cockerels fed PCB's also displayed decreased voltage, but no change occurred in the mean electrical axes, and attributed the voltage decrease to hydropericardium.

Although inanition of the pair-fed controls had no effect on the mean electrical axes, there was a significant change in the RS complex voltages relative to those of the ad libitum control birds (Tables 6 and 7). Since ECG amplitude varies directly with the mass of depolarizing myocardium, the pair-fed cockerels may have exhibited smaller RS complex voltages due to decreased heart weights as the result of limited feed intake. In support of this, Kowaleski (1976) found that the heart weights of cockerels pair-fed to the feed consumption of PBB-fed individuals were significantly decreased.

SUMMARY AND CONCLUSIONS

1. Single Comb White Leghorn (SCWL) cockerels fed rations containing 75 and 150 ppm of polybrominated biphenyls (PBB) in the form of FireMaster FF-1 exhibited decreased weight gains. The reduction in weight gains was primarily the result of decreased feed consumption among cockerels receiving rations containing PBB's. Since the body weights among cockerels in the group fed the 150 ppm PBB rations were significantly less than those of their pair-fed controls, PBB's must have had an additional effect on weight gain which was not attributable to the decrease in feed intake.

2. Hematocrits were decreased significantly (13.7%) in the group fed rations containing 150 ppm PBB relative to those of the pair-fed control group after 5 weeks of consuming the experimental diets. After 9 weeks the hematocrits of the cockerels fed the 75 and 150 ppm PBB rations were decreased relative to the hematocrits of the pair-fed controls (20.4% and 31.7% decrease, respectively). After being fed the experimental diets for 9 weeks the hemoglobin concentrations of the 75 and 150 ppm PBB treatment groups were decreased 18.6% and 33.0%, respectively, relative to those of the pair-fed controls. Since pair-feeding had no effect on the hematocrits and hemoglobin concentrations of the pair-fed birds, the decrease in

these parameters among PBB-fed cockerels was directly attributable to PBB's.

3. Cardiac output ($\dot{Q}$), cardiac index and heart rate decreased in the 150 ppm PBB treatment group with respect to its paired control group. Cardiac index and $\dot{Q}$, but not heart rate, decreased significantly in the 75 ppm PBB treatment group. Concurrently, no statistically significant change occurred in stroke volume. Heart rate and $\dot{Q}$ were positively correlated, indicating that the decreased cardiac output found in the PBB-fed cockerels resulted from bradycardia.

4. Total peripheral resistance (TPR) increased in the group fed rations containing 150 ppm PBB. Since mean arterial pressure of this group did not differ significantly from that of the paired control group, despite a decreased $\dot{Q}$, TPR may have increased as a result of reflex vasoconstriction in an attempt to maintain blood pressure homeostasis.

5. Although the PBB-fed cockerels which died prior to the end of the experiment appeared to have slow and labored breathing, no statistical differences were found among the average respiratory rates of the experimental groups. The respiratory difficulties observed in the dying PBB-fed cockerels may have been related to the muscular weakness noted shortly before death occurred.

6. Hydropericardium was statistically significant only among cockerels fed the 150 ppm PBB rations. The changes in the fluid dynamics of the pericardial space which brought about the increased pericardial fluid volume are not known,

but could include increased capillary permeability, increased capillary hydrostatic pressure, decreased plasma colloid osmotic pressure or decreased lymphatic drainage. The development of hydropericardium was due to PBB's and was not related to inanition since pair-fed control cockerels exhibited normal pericardial fluid volumes.

7. Changes occurred in the electrocardiograms (ECG's) of PBB-fed cockerels. Lead II S wave voltage and RS complex voltage decreased relative to those of the pair-fed and ad libitum controls. The decreased voltages were due to increased pericardial fluid volumes in the PBB-fed birds and, possibly, to decreased myocardial mass. The mean electrical axes of cockerels fed rations containing 150 ppm PBB were more positive than those of the controls, possibly due to degenerative changes in the myocardium. With the exception of the decreased RS complex voltages, the changes in ECG's were not attributable to feed restriction. RS complex voltages in leads II and III of the ECG's of pair-fed control cockerels as well as those of the PBB-fed cockerels were less than those of the ad libitum controls, indicating that at least part of the decrease seen in ECG voltages of PBB-fed birds was due to the restriction of feed intake.

8. The effects of inanition among the cockerels pair-fed to the PBB treatment groups included decreased body weight, increased mortality, increased cardiac index and decreased RS complex voltages in leads II and III. The most likely cause of the decreased body weight and increased mortality among the

pair-fed birds was the lack of sufficient feed. Similarly, the decreased voltages in the RS complexes were probably due to a decrease in the ventricular mass as a result of the limited feed intake. The increase in the cardiac indices of the pair-fed cockerels cannot be explained on the basis of the results of this study. Further investigation is needed to determine the mechanism through which feed restriction altered the cardiac index.

Parameters which did not change in the pair-fed control groups relative to the ad libitum control group but did change in the PBB-fed groups were hematocrit, hemoglobin concentration, heart rate, pericardial fluid volume, total peripheral resistance, mean electrical axis and S wave amplitude. The lack of statistically significant differences among the pair-fed controls and the ad libitum control, despite the existence of significant differences between the PBB treatment groups and the control groups, indicated that the effect of PBB's on these parameters was not attributable to the restriction of feed intake, but to a direct effect of PBB's.

APPENDICES

APPENDIX A

CHEMICAL NAMES OF INSECTICIDES

APPENDIX A

CHEMICAL NAMES OF INSECTICIDES

Aldrin - 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-endo-exo-1,4:5,8-dimethanonaphthalene.

DDD - 6,6'-Dithiodi-2-naphthol.

DDT - 1,1,1-Trichloro-2,2-bis(p-chlorophenyl)-ethane.

Dieldrin - 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-exo-1,4: 5,8-dimethano-naphthalene.

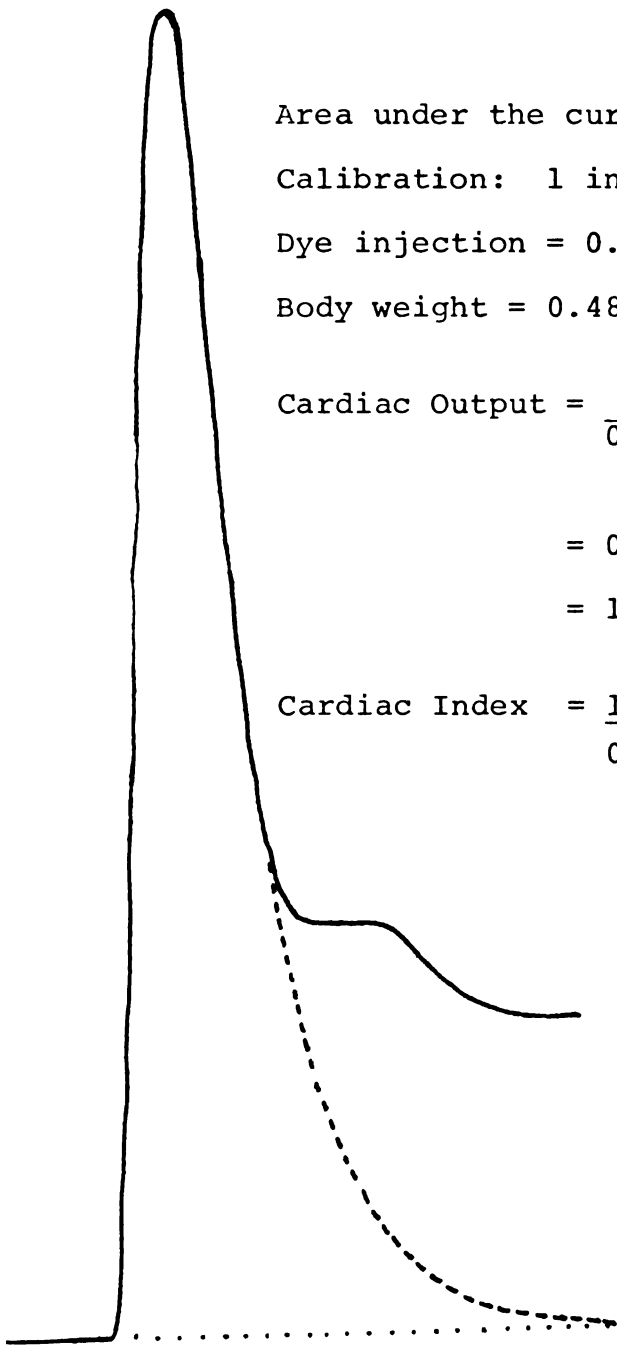
Endrin - 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-endo-1,4:5,8-dimethano-naphthalene.

APPENDIX B

SAMPLE CALCULATION OF CARDIAC OUTPUT AND CARDIAC INDEX

APPENDIX B

SAMPLE CALCULATION OF CARDIAC OUTPUT AND CARDIAC INDEX



Area under the curve = 4.513 $inches^2$

Calibration: 1 $inch^2$ = 0.0886 mg minutes/liter

Dye injection = 0.07 mg

Body weight = 0.480 kg

$$\text{Cardiac Output} = \frac{0.07 \text{ mg}}{0.0886 \frac{\text{mg minutes/liter}}{\text{inches}^2} \times 4.513 \text{ inches}^2}$$

$$= 0.175 \text{ liters/minute}$$

$$= 175 \text{ ml/minute}$$

$$\text{Cardiac Index} = \frac{175 \text{ ml/minute}}{0.480 \text{ kg}^{0.734}}$$

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in PBB-fed cockerels. It can be stated that the bradycardia was not due to inanition since the heart rates of the pair-fed controls were normal.

The immediate determinants of cardiac output ($\dot{Q}$) are stroke volume and heart rate. Stroke volume did not differ significantly among the experimental groups (Table 4). Bradycardia was statistically significant in the 150 ppm PBB treatment group. The correlation between $\dot{Q}$ and heart rate was also statistically significant ($r=0.646$), indicating that $\dot{Q}$ was decreased primarily due to the decreased heart rate. It was also possible that the large amount of pericardial fluid in PBB-fed cockerels could have influenced $\dot{Q}$. Fluid accumulation in the pericardial space can reduce $\dot{Q}$ by exerting pressure on the ventricles, thus decreasing the end diastolic volume and ultimately decreasing the stroke volume (Dodge and Kennedy, 1974). The decrease in $\dot{Q}$ (Table 5) was not, however, correlated with the increase in pericardial fluid (Table 2) among the PBB-fed cockerels ($r=0.006$). Evidently, the transudate accumulated at a slow enough rate to allow the pericardium to stretch, thus avoiding cardiac tamponade.

The cardiac indices (Table 5) of the pair-fed controls were significantly increased over those of the ad libitum controls as well as those of the PBB-fed groups. Although the ad libitum control group's mean cardiac output was not significantly less than those of the pair-fed control groups, there was a trend for the pair-fed controls to have greater cardiac outputs. Apparently, restriction of feed intake stimulated

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