INFLUENCES OF POLYCHLORINATED BIPHENYL ADMINISTRATION ON REPRODUCTION AND THYROID FUNCTION IN MINK (MUSTELA VISON)

Dissertation for the Degree of Ph. D.
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Robert K Ringer

Major professor

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ABSTRACT

INFLUENCES OF POLYCHLORINATED BIPHENYL ADMINISTRATION ON REPRODUCTION AND THYROID FUNCTION IN MINK (MUSTELA VISON)

By

James J. Byrne

Studies were designed to determine the effects of long-term feeding of a polychlorinated biphenyl (PCB) on thyroid function and reproduction of female mink. Plasma thyroxine (T_4) was assessed over a 9 month period which included the reproductive season in 32 mink fed PCB Aroclor® 1254[1] levels of 5 ppm, 2 ppm, 0.5 ppm and untreated controls. A second group of 16 mink fed 5 ppm PCB plus controls had quantitative measurements of thyroid function assessed at diestrus, midgestation and lactation using the ^{131}I-thyroxine degradation method for estimating thyroxine secretion rate (TSR), biological half-life ($t_{\frac{1}{2}}$), thyroxine degradation rate constant (K), thyroxine distribution space (TDS/100 gm b.w.) and extra thyroidal thyroxine (Ett). Plasma thyroxine (T_4), thyroxine binding globulin-capacities (TBG) and saturation index (SI) were also measured at the same three dates.

PCB generally increased T_4 levels and peripheral degradation of thyroxine except during estrus and pregnancy.

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Mink fed at the two highest PCB levels were relatively hypothyroid only at estrus and the reproductive season. They failed to bear young. The PCB fed at 0.5 ppm consistently increased T_4 levels above those of the controls in which large increases in T_4 were observed during estrus, implantation and early pregnancy. Birth rate and weaning rate were significantly higher in the 0.5 ppm PCB group than in the controls. Birth rate was directly correlated to relative T_4 levels during estrus, implantation and early gestation.

TBG-capacity changed little in the PCB-treated animals but apparent binding to other T_4 binding proteins did increase as shown by a large increase in the saturation index.

Liver weights and adrenal weights were significantly higher in the 5 ppm PCB group than in controls. Thyroid weight was nonsignificantly higher in the 5 ppm PCB group than in the controls. Histologically, the thyroid revealed anatomical evidence of stimulation in the 5 ppm PCB group.

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[1]Aroclor®1254--Trade name for PCB containing 54% chlorine, Monsanto Chemical Company, St. Louis, Missouri.

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INFLUENCES OF POLYCHLORINATED BIPHENYL ADMINISTRATION ON REPRODUCTION AND THYROID FUNCTION IN MINK (*MUSTELA VISON*)

By

James J. Byrne

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1974

Dedicated to my wife Leslie for her patience, understanding and invaluable support throughout this program, including preparation of this dissertation.

Dedicated also to my children Kim and Rich.

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INTRODUCTION

The thyroid gland affects the structure and function of nearly every tissue within the body. Thyroid hormones have an effect on both major control systems--the nervous system and the endocrine system. Both systems are dependent upon thyroxine complementation for their "target" tissue integrity and also for their own cellular integrity. Thyroid hormones are involved in major biological events such as growth and development, basal metabolism, temperature regulation, reproduction, lactation and numerous intracellular events upon which the gross events depend.

Because of the many bioeffects of thyroid hormones, estimations of thyroid function are an excellent index of an animal's well-being.

During the brief history of endocrinology several methods have been used in an attempt to establish reliable indices of thyroid function. Among them thyroid secretion rate, serum thyroxine, thyroxine degradation rates, thyroxine-binding capacity of T_4 binding proteins, protein bound iodine and thyroidal uptake of iodine have been the most successfully employed. Effects of external factors on the thyroid when mirrored by the indirect estimates of thyroid function provide insight into the probable effects

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upon the body of a treatment and its associated biological events. Because the thyroid is so essential in modifying the major systems, any factor suspected of affecting its function or that of its hormones should be investigated. Polychlorinated biphenyls (PCB's) are one such factor.

PCB's have been implicated in morphological changes in the thyroid gland of lower animals and are known to affect liver enzymes in diverse species. Although PCB's chemically resemble such pesticides as DDT, DDE and dieldrin they are used for quite different purposes and their physiological effects are probably not identical.

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REVIEW OF LITERATURE

I. Polychlorinated Biphenyls

During the last three decades the world has been undergoing a synthetic chemical revolution. Large quantities of synthetic organic compounds have been discharged into the biosphere. Reliance has been placed on dilution and degradation to dispose of them, but with insufficient knowledge of their natural degradation rates. Within the last half dozen years the production of these chemicals has exceeded their dilutability. Many of the man-made chemicals have effects far beyond their original intent. Many have biological and toxicological effects upon vertebrates as well as invertebrates. The ability to synthesize and put these chemicals into use has by far exceeded the research into their apparent multifold biological effects. For a number of years research centered around DDT, DDE, dieldrin and related pesticides which are naturally suspected to cause environmental problems. Most recently the scope of the investigations has been enlarged, and new culprits and many new suspects are now being investigated. Polychlorinated biphenyls (PCB's) along with other halogenated biphenyls comprise such a group.

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PCB's have found their way into the environment by many routes. They are transported throughout the ecosystem both aquatically and terrestrially. Their properties of lipid solubility and chemical solubility have contributed to PCB's ubiquitous presence in the environment and allows them to enter easily into food chains.

Polychlorinated biphenyls, as the name implies, are comprised of a biphenyl (2 covalently bonded benzene rings) with chlorine substitutions. There are ten possible sites for attachment of chlorine; however, anywhere from four to eight of the available carbons may be chlorinated (Jensen, 1970). With four to eight sites available there is a potential for 102 different isomers.

The environmental hazard of a chemical does not necessarily lie in its benzene ring structure and chlorine atoms alone (Bitman and Cecil, 1970). Dustman et al. (1971) pointed out that DDT, DDE, and methoxychlor (see Appendix A) all have similar basic structures (dibenzene) but methoxychlor is rapidly broken down and is rarely found in mammals. It seems that toxicity of a chemical is then a function of its specific structure not necessarily the quantity of benzene, chlorine or carbon present.

Identification of PCB's in the environment came about slowly. Part of the problem was the confounding of PCB's with known pesticides, and also because PCB's are comprised

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of many different compounds and isomers which produce many different peaks upon gas chromatography and spectrographic analysis. Albro and Fishbein (1972) and Zitko & Choi (1972), reported that variation in detection response to each component on chromatograms complicated the number of peaks present in quantitative analysis for PCB's. Although a specific mixture of PCB's will elicit a given set of peaks, any variation in the composition will unpredictably change the total peak response. This problem becomes further complicated when analyzing biological material for PCB's for the mixtures are variable and random and are not easily matched with commercial PCB mixtures. Simmons and Tatton (1967) reported that organochlorine pesticides produce gas chromatographic peaks which coincide with the peak produced by PCB's, further complicating analysis for PCB. DDT and DDE have peaks very much like PCB's. Soren Jensen (1966) while looking at DDT and DDE was the first to identify PCB's as a "troublesome unknown" which some previous authors had reported but had not identified. The separation of other organochlorides from PCB's was done by several methods Simmons and Tatton, 1967; Holden and Marsden (1969). It is more difficult than analysis for organochloride, by gas chromatography (Risebrough et al., 1969) and by thin-layer chromatography procedures (Mulhern et al., 1971).

Mass spectrography was the first technique used to confirm and identify a given gas liquid chromatographic

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(GLC) peak as due to a PCB and determine the number of chlorine atoms per molecule. Jensen (1966,1970), was the first to identify PCB's in biological tissue. Koeman et al. (1969), using Jensen's method, affirmed PCB presence in Norwegian wildlife. Stalling (1971) used the mass spectrograph but preceded it with a chromatograph to effect a separation of the various PCB isomers. Bagley et al. (1970), identified 18 PCB isomers and Biros et al. (1970) identified PCB's in human tissue and hair. Both used mass spectrography. Confoundment of DDT by PCB's is not simply a function of their equally widespread distribution but recent studies by Maugh (1973) have shown that irradiation of DDT vapor using ultra violet light, approximating sunlight at ambient intensity, precipitates conversion of DDT to PCB. The major step of this organic transformation seems to be the removal of the ethane group from between the benzene rings (see Appendix A for comparative structures).

Polychlorinated biphenyls were first described by Schmidt and Schultz in 1881 (Peakall and Lincer, 1970) and were first introduced in 1929 as flame resistant electrical transformers and conducters (Dustman et al., 1971). Penning in 1930 (Peakall and Lincer, 1970) expounded on their physical and commercial characteristics and additional practical applications. Since that time and particularly since World War II the use of various PCB's has expanded in

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explosive proportions. They are widely used as plasticizers, in all types of plastics, heat transfer agents, hydraulic fluids, protective coatings (Rhee and Plapp, 1973) marine antifouling paints, in cardboard cartons (Bailey et al., 1970), as "inert" ingredients or carriers for insecticides, as dustallayers in detergents, and as vapor suppressors in spray compounds, carbon paper, mimeograph fluids and typing ink.

PCB's are highly valued for their qualities of viscosity, heat conduction, water resistance, flexibility, and adhesion properties. They are marketed and manufactured by companies throughout the world. Monsanto is the major United States producer under the tradename Aroclor. France produces Clophen and Japan produces Kanechlor. Great Britian, Italy, USSR, and Czechoslovakia also have PCB manufacturers. PCB's are marketed using differing percentages of chlorine depending primarily on the purpose. An increase in percent chlorine increases the viscosity of the PCB. Above 60% chlorine content, the PCB's pass from the amorphous state to a pliable resin. Monsanto's Aroclor is designated by a 4 digit number, the first two digits are a trade designation, the second two digits indicate the percent by weight of chlorine. For example, Aroclor 1254 contains 54% chlorine. The range of chlorine in commercially available chlorinated biphenyls is from 28% to 68%.

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The United States produces hundreds of millions of pounds of PCB's annually and because few product manufacturers list the ingredients of their products, PCB uses are not well-known. Production secrets also contribute to some of the mystery surrounding this widely sold product. The particular environmental disadvantage of PCB's is a result of their lipid solubility and their low degradability, both of which contribute to their longevity and increased concentration of the food chain.

PCB's find their way into the environment in numerous ways, many of which are extremely difficult to trace; however, with their widespread use it is easy to guess some of the routes of contamination. The major cause is probably slovenly handling of individual, industrial and municipal wastes. In several areas of the world, PCB contamination has been traced back to this source. Koeman et al. (1969), in the Netherlands, found that PCB's present in seabirds and fish on the European Atlantic coast came from discharge of the highly polluted Rhine River. Duke et al. (1970), in the United States, found the source of mollusk and fish PCB contamination at a factory which was leaking PCB's into the river. Holden (1970), on Scotlands Atlantic coast, found that sludge from sewage treatment plants which was being dumped in a deep water estuary contaminated shellfish and marine life nearby. PCB's have been detected in both raw and processed foodstuffs (Gustafson, 1970) and in milk

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The incidence of PCB's and other halogenated biphenyls resulting in contamination of animal species, including man are both numerous and world-wide. The following are a few of the species contaminated: Fish and sea life (Jensen, 1966; Jensen et al., 1969; Koeman, 1969), brook trout (Hutzinger et al., 1972), sediment and biota of lakes (Duke et al., 1970), cormorants, pelicans (Anderson et al., 1969), pheasants (Dahlgren et al., 1971), bald eagles (Mulhern et al., 1970; Reichel et al., 1969), ducks (Friend & Trainer, 1970), seals and porpoises (Holdin and Marsden, 1967), cattle and swine (Platonow & Tunnel, 1971; Platonow et al., 1972), mink (Aulerich et al., 1972) and in human adipose tissue (Biros et al., 1970).

Toxicology of PCB's

Toxicity of PCB's to industrial workers has been known for a number of years. Jones and Alden (1936), Schwartz (1936) and Meigs et al. (1954) described a disease named chloracne which was an occupational disease of PCB-industry workers. Crow (1970) and Kuratsune et al. (1972) reported that consumption of rice-bran oil accidently contaminated with PCB's produced the disease "yusho" in Fukuska-Ken, Japan. The most common symptoms were eye discharge,

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follicular accentuation, acne-form eruptions, sweating palms, weakness and pigmentation of the skin and nails.

It was not until recently, however, when it was found that PCB's are toxic at much lower concentrations than had been previously imagined. Voss and Notenboom-Ram (1972) administered PCB's topically to rabbits upon a small shaved area of approximately 5 by 10 cm. After 4 weeks of Aroclor 1260 (20 applications of 120 mg PCB) microscopic examination of the liver showed degeneration of some cell membranes and cytoplasm with decreased glycogen stores and damaged endoplasmic reticulum. Liver weight also increased and acne (chloracne) was noticed.

Heath et al. (1972) tested in birds 6 PCB mixtures ranging from 32 to 62 percent chlorine. Although each species had a specific sensitivity to PCB's, increased toxicity was associated with increased chlorine percent in the mixtures. Toxicities of PCB were also similar to those of DDE. Bobwhite quail were most sensitive, followed in turn by pheasants, mallards, and coturnix. Birds became lethargic during PCB administration and tended to assume a crouching position. They displayed mild tremors during the last hours of the experiment. Heath et al. also found that the toxic effects of PCB 1254 were additive to those of DDE when fed concurrently. Aulerich et al. (1973) and Platonow and Karstad (1972) reported that very low levels of PCB (below 5 ppm) resulted in mortality in mink fed PCB's for

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Prestt et al. (1970) found that PCB 1254 was about one-third as toxic as DDT in Bengalese finches. Dahlgren et al. (1972) found that the degree of PCB sensitivity in a species was variable and depended upon the individual animal. For example, some birds died 30 days following a 10 mg per day dose while a few birds of the same species lived until sacrificed. Eight months later they noted that brain tissue levels of PCB's were much more useful for diagnosing cause of death than either muscle or liver levels. They reported that brain PCB residues are 300 to 400 ppm in a majority of deaths, which suggests a range or level to use as an index of PCB-induced mortality.

Fish → Hattula and Karlog (1972) studied veil-tailed goldfish in aluminum-lined aquaria which continuously contained from 0, 0.5, 1.5, 2.0, 2.5 and 4.0 ppm Clophen A 50 (Bayer) (50% chlorine). When PCB concentration was regressed against time on a semi-log scale the 50% mortality was linear and had a highly correlated negative slope. The LD_{50} at 20 days was 0.5 ppm PCB 1254 and the LD_{50} at 5 days was 4.0 ppm PCB 1254. Harmful effects of PCB were easily observed. The bright orange color turned pale yellow and the fish lost their appetites. Nervous system effects were primarily uncoordinated movements.

Fish

Rabbit Koller and Zinkel (1973) administered PCB's 1221, 1242, and 1254 once each week for 14 weeks to adult rabbits.

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Livers of the 1254- and 1242-treated rabbits were significantly enlarged compared to the 1221 group and controls. Megalohepatocytosis and necrosis of the midzone of the liver was observed in animals on the two highest treatment levels. Fibrous connective tissue filled the necrotic zones. Rough endoplasmic reticulum in the 1254 group livers appeared to have been destroyed. Uteri of the 1254 group had also atrophied.

Lichtenstein et al. (1969) found that in Dipteran insects (flies) PCB's were toxic but percent chlorination was inversely related to mortality which is opposite that found in birds. The toxic effects of PCB's above 48% are very low in house flies. PCB toxicity was more than just additive to those of DDT or dieldrin. Rhee and Plapp (1973), found that induction of microsomal enzymes of some strains of house flies was directly proportional to the percent chlorine in the PCB.

Crustaceans and mollusks are extremely sensitive to PCB's. Duke et al. (1970) found that 48 hour exposure to 100 ppb of Aroclor 1254 was a lethal dose for shrimp. In 24 hours 80% were dead. Accumulation of 3.9 ppm PCB was found in the tissues, but in shrimp accumulating 1.3 ppm PCB at a treatment level of 10 ppb none died. Exposure for 20 days at 5 ppb Aroclor 1254 killed 72 percent of juvenile shrimp even though the tissues had accumulated 16 ppm. Crabs were less sensitive for they received 5 ppb for 28 days

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and accumulated 23 ppm but did not die. Oyster shell growth was completely stopped after 96 hours at 100 ppb, but a 10 fold reduction in PCB only reduced shell growth to 40% of normal.

Tissue cell cultures from Chinese hamster (quasi-diploid epithelial cells) were found to be most sensitive to PCB 1016 which was a distillate of PCB 1254. As the percent chlorine was decreased, toxicity increased. In human lymphocyte cultures Aroclor 1254 at 100 ppm caused no apparent effect upon chromosomal integrity as measured by cytological evidence (Hoopingarner et al., 1972).

Fetotoxicity of PCB's

Fetotoxicity of PCB 1254 has been demonstrated by Villeneuve et al. (1971). Doses of 12.5 to 50 mg/kg/day, administered orally, induced abortions and were toxic to rabbit fetuses during the first 28 days of gestation. Doses of 10 mg/kg/day were insufficient to induce abortion. Aroclor 1221 at up to 25 mg/kg/day produced no fetotoxic effects. Dead fetuses whose mothers had received 12.5 mg/kg/day showed no skeletal abnormalities. Rats treated with up to 100 mg/kg/day PCB 1254 did not have fetal deaths nor malformations. McLaughlin et al. (1963) injected PCB 1242 into chicken eggs at concentrations of 10 and 25 mg and found that growth was retarded, beak development was affected and they had only 0 to 5% hatchability.

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Rabbit placental transfer

Grant et al. (1971) found that PCB's 1221 and 1254 crossed rabbit placentae when administered orally during gestation. There was also a direct correlation between amounts of PCB given and the amount concentrated in the liver and fat depots of fetuses and the does. Fetal liver had much higher concentrations of PCB than the doe's liver. Aroclor 1254 accumulated to a much greater extent than did Aroclor 1221. The difference in fetal and doe liver levels might be a result of the fetus not possessing the full complement of enzymes necessary for degradation of PCB's. Villeneuve et al. (1971) found that adult and fetal livers did not differ in protein or carboxylesterase when dosed for 28 days with 0, 1, or 10 mg/kg/day of Aroclor 1221 or 1254; however, 10 mg/kg/day of Aroclor 1254 induced microsomal enzymes (carboxylesterase, aniline hydroxylase and aminopyrine n-demethylase) in the dam (Grand et al., 1971). Eckhoff (1972) reviewed the mechanisms of transplacental passage of drugs and exogenous compounds and indicated that most exogenous molecules, particularly lipid soluble ones, cross the placenta by simple diffusion. Platanow and Chen (1973) reported transplacental transfer of PCB 1254 in the cow and fetal PCB concentrations were higher than maternal only in kidney tissue.

Cow placental transfer

Reproductive Effects of PCB's

PCB's were first shown to affect reproduction in mammals by Gilbert (1969) and later by Aulerich et al.

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(1971), both in mink which had been fed PCB-contaminated fish. Ringer et al. (1972) fed PCB's to mink in approximately the same levels as those contained in Lake Michigan Coho salmon (10 ppm), demonstrating and confirming previous suspicions that PCB's were a causative agent in reproductive failure of mink.

Orberg & Kihlstrom (1973) and Kihlstrom et al. (1973) found that PCB as well as DDT significantly lengthened the estrous cycle of rodents and suppressed the appearance of vaginal cornified epithelial cells. In an experiment using mice, Kihlstrom et al. (1973) found that adult mice which had been suckling PCB-contaminated milk as neonates, when mated, demonstrated a significant decrease in the frequency of implanted ova. Adult rats also maintained on PCB (Clophen A 60, 20 mg/kg body weight) showed a decrease in implanted ova. These studies imply an estrogenic effect of PCB's since any alteration in the sensitive balance of estrogen and progesterone would interfere with either implantation or estrus or both.

Bitman and Cecil (1970) suggested that the geometric similarity of PCB and DDT to the synthetic estrogen diethylstilbestrol (DES) may be functional as well as structural. Using the sensitive 18 hour glycogen response of the rat uterus, they found that DDT is about 1000 times less estrogenic than natural estrogen. They found that active estrogenicity is dependent upon the presence of at

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least one phenolic hydroxy ring structure. PCB's were found to be at least as estrogenic as DDT. PCB's of lower chlorination (21-48 percent) were more estrogenic in rats than the higher chlorinated (54-68 percent).

In addition to being naturally estrogenic in nature PCB's affect enzyme systems which govern steroid levels. Plantnow et al. (1972) have found that PCB's markedly reduce the urinary excretion level of gonadal steroids in subjects receiving daily oral doses of PCB (1254, 10 mg/kg b.w.) and receiving a single oral dose (100 mg/kg b.w.). PCB's also have the capacity to enhance steroid-hydroxylating enzyme activity in the liver. Risebrough et al. (1968) demonstrated that PCB enzyme induction increased estradiol metabolism in pigeons. Peakall and Lincer (1970) reported that PCB increases in vitro metabolism of estradiol and also increases cytoplasmic RNA in birds (American kestrels) which had been maintained on 0.5 and 5 ppm PCB.

Birds have been found to be particularly sensitive to PCB's. Rehfeld et al. (1971) and Plantnow and Funnel (1972) have reported that PCB's inhibited secondary sexual characteristics in chickens which was manifested in reduced comb and testicular development. Kolbye (1972) reported that PCB-contaminated chicken eggs had alarmingly reduced hatchability. Data from Plantnow and Reinhart (1973) showed that the concentrations of PCB in eggs inversely reflects the rate at which eggs are produced. When 50 ppm PCB (1254) are

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added to the diet, hatchability is severely affected but at 5 ppm PCB, no effect is seen on hatchability of fertile eggs. When PCB levels surpassed 15 ppm, an instantaneous depression in hatchability was noted. Embryonic mortality occurred mainly in the latter stages of incubation during the first 2 weeks PCB's were fed to hens. After 2 weeks, embryo mortality occurred mainly in the early embryonic stage.

Heath et al. (1972) reported that Aroclor 1242 affected reproduction of White Leghorns at 10 ppm, but much higher dosages were required to alter reproduction (100 ppm). Neither egg shell thickness nor reproduction were affected in Mallards or Bobwhite quail receiving 25 ppm and 50 ppm (1254).

Peakall (1971) was first to report the lack of effect of low level PCB upon egg shell thickness. Anderson et al. (1969) in cormorants and wild pelicans reported that DDE residues in eggs correlated better with shell thinning than did residues of PCB or dieldrin. Dustman et al. (1971) reported evidence from the Industrial Bio-test Laboratories which was conducting research for Monsanto Chemical Company. They found Aroclor 1254 at 100 ppm and Aroclor 1242 at 10 ppm or 100 ppm in the diet of chickens, caused thinning of eggshells and reduced egg hatchability and production. The information concerning the effects of PCB's upon bird reproduction, egg production, hatchability and shell strength is

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not simple or clear-cut. The following four factors are the determinants of PCB function: 1) which PCB is used, 2) amount of PCB used, 3) species of test animal, 4) duration factor of administration.

PCB's and Thyroid Function

Very recently, PCB's have been implicated in avian thyroid weight and ^{131}I uptake. Jeffries and Parslow (1972) reported that thyroid weights of black billed gulls which had been reared in pens and received daily dosages of 50, 100, 200, 400 mg/kg 1254, increased significantly from a mean of 30.5 $\pm$ 1.81 mg to 40.28 $\pm$ 2.31, an increase of 32% over controls. At these four high dosages, no difference in effect from one PCB dosage to the next was observed. They also reported that thyroid follicles were larger and contained more colloidal space per follicle. This is different from DDT treated animals which have almost complete colloidal loss and hyperplasia. The PCB-induced colloidal increase amounted to a 22% increase in area. Most recently Hurst et al. (1974) found that Bobwhite quail responded to PCB Aroclor 1260 by a decrease in thyroid size at low dosage levels and to stimulate thyroid growth at high dose levels (dosages 5, 50, 500 ppm). The enlarged glands of the highest PCB group took up more ^{131}I than controls but when calculated on the basis of thyroid weight, no difference was observed. Thyroids of the two lowest level PCB groups took

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up the same amount as the controls but each unit weight of thyroid tissue took up less ^{131}I. DDT and toxaphene stimulated ^{131}I uptake in the Bobwhite and increased thyroid weight. Channel catfish have also been known to increase thyroid size and ^{131}I uptake upon introduction of PCB (1254) into the water (Mayer et al., 1972).

In addition to the numerous reports of PCB-caused liver weight increases, PCB's also have been implicated in liver enzyme induction. Risebrough et al. (1968) and Peakall and Lincer (1970) have been cited earlier for their work on steroid-hydroxylating enzyme and for in vitro estradiol metabolism by liver tissue. Benthe et al. (1972) reported that PCB's and stress stimulate rat liver microsomal drug-oxidizing enzymes and that tetrachlorobiphenyls are distinctly more effective enzyme inducers than dichlorobiphenyls. Rhee and Plapp (1973) found that PCB 1254 was a more effective inducer of the microsomal enzyme aldrin epoxidase than 1242 and 1221 in the house fly. The rate of induction decreased with decreasing percent chlorine. They have also pointed out that differences among house fly strains exist in 1) dose dependency, and 2) the time course of enzyme induction.

Liver microsomal cytochrome P-450 complex was significantly increased by Aroclor 1254 (Konat and Clausen, 1973) in mice. Mixed function oxygenase was also increased. PCB 1254 stimulated proliferation of liver endoplasmic

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reticulum as ascertained by the evaluation of increased microsomal protein content. Both lindane and PCB 1254 stimulated liver microsomal esterase. Aroclor 1254 was found to be a better general stimulator of liver toxin metabolism than lindane, and DDT was the least stimulatory. PCB's were found not to change brain cytoplasmic esterases but to act primarily on liver enzymes.

Storage and Elimination of PCB's

PCB 1254 was found to be concentrated in the liver of the pheasant (Dahlgren et al., 1971) after feeding of Aroclor 1254. The second and third highest levels were found in brain and muscle,respectively. Administration of Aroclor 1254 as a 50 mg capsule resulted in a 94% absorption during the first 24 hours and of the PCB absorbed, 410 mg was excreted in feces and 4.2 mg was excreted in the eggs. In pheasants receiving a single 50 mg capsule of 1254, 1.5 mg PCB was found per egg 2 weeks after administration. A total 40.5 mg of a single 50 mg PCB dose was found by whole body analysis after 24 days. In mink, Plantnow and Karstad (1972) found that PCB's were concentrated at the highest level in the liver and lowest in blood. Heart usually had more than skeletal muscle.

Analysis of tissue extracts for Aroclor 1254 in the Bobwhite quail, using mass spectrography, was found by Bagley and Cromartie (1973) to contain all the relative peak heights associated with Aroclor 1254, suggesting that

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all components of the Aroclor were readily absorbed. Fourteen days after Aroclor 1254 feeding a methodical elimination of certain components and an increase in others was observed. Their mass spectroscopic (MS) findings were confirmed using gas liquid chromatography (GLC). The chromatographs show that a dynamic system was at work completely removing some chemical components while increasing others. No foreign chlorinated isomers were observed, but isomerization of Aroclor 1254 is strongly suggested by the changing MS and GLC peaks. Grant et al. (1971) in a study of Aroclor 1254 metabolism in male rats found that GLC-electron capture patterns of the PCB residues were significantly different from those of the standards.

Bagley and Cromartie (1973) reported that after 42 days post-treatment components of Aroclor 1254 were hardly in evidence but several large peaks were observed. Mass spectrographic analysis showed butyl esters of short-chain fatty acids. It was thought that these were thermal degradation products. They were observed only in PCB-treated birds. Bagley and Cromartie (1973) suggested that they might be related to the "fatty degeneration" observed by Vos and Koeman (1970).

Biros et al. (1970), reported that human adipose tissue samples (2 humans) examined by combined gas chromatography-mass spectrometry were found to contain substantial quantities of PCB's ranging from pentachlorobiphenyl to

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decachlorobiphenyl including at least 14 isomers and homologs; however, the origin of the PCB compounds was not known.

Metabolism and excretion of pure PCB's was undertaken by Hutzinger et al. (1972). They administered mono-, di-, tetra-, and hexachlorinated biphenyl isomers to pigeons, rats and brook trout. They found that excreta when examined by chromatographic and mass spectrometric techniques showed conversions of 4 chloro-, 4,4'-di-chloro and 2,2',5,5'-tetrachlorobiphenyl isomers into monohydroxylated derivatives by rats and pigeons but brook trout excreta contained no hydroxymetabolites. None of the excreta of species examined contained 2,2,4,4',5,5'-hexachlorinated biphenyls. This study confirmed the suspicion that PCB's are eliminated in forms other than those injected.

Effects of PCB's on Organ Weight and Function

Liver weight has been shown in numerous studies to increase as a function of PCB treatment, particularly with Aroclor 1242 and 1254 (Grant et al., 1971; Rehfeld et al., 1971; Platonow and Funnel, 1971; Lincer and Peakall, 1973; Bitman et al., 1972; Cecil et al., 1973; Abrahanson and Allen, 1973; Dahlgren et al., 1972; Voss and Beems, 1971).

The effect of PCB's in other organs is not so clear-cut. The following organs were found to have weight changes with PCB administration: The heart weight decreased

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(Dahlgren et al., 1972; Iturri, 1974), spleen weight decreased (Dahlgren et al., 1972; Flick et al., 1965; Grant et al., 1971), adrenal weight increased (Flick et al., 1965), kidney weight increased (Dahlgren et al., 1972; Prestt et al., 1970), testis weight decreased (Platonow and Funnel, 1971).

In a most recent study by Iturri (1974) PCB's were implicated in cardiovascular and hematological problems in birds. PCB's 1242 and 1254 at 100 ppm reduced heart rate and the former also decreased blood pressure. Two PCB's also produced abnormal electrocardiograms in White Leghorn cockerals (inverted T-waves, prominent T or P waves and lower S waves). Hematocrit, hemoglobin concentration and total erythrocyte concentrations were decreased and the two PCB's also produced observable anemia. Arterial blood pH was found to decrease (1254) but plasma K+ concentration was significantly higher (1242). Although Na+ did not change in arterial blood, both Na+ and K+ concentrations were increased in pericardial fluid. Hydropericardia was also observed without producing bradycardia.

II. Thyroid Function

Thyroid Secretion

Estimation of thyroxine secretion rate (TSR) has been attempted in vivo in numerous manners on many varied animal

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species. Bioassay technique by the goiter prevention method was used first by Dempsey and Astwood (1943) and by Mixner et al. (1944). In this method a goitrogen is administered to test animals; this suppresses thyroid hormone formation and compensatorially results in an increased thyroid stimulating hormone (TSH) release. Increased TSH then stimulates thyroid growth. However, known quantities of thyroxine plus unknowns can be administered to suppress TSH release and the resultant change in thyroid weight of the goitrogen-receiving animal can be compared with the controls. The daily dosage of thyroxine to animals receiving the goitrogen which results in thyroid weight equal to the untreated groups is assumed to be the daily rate of thyroxine secretion.

A thyroxine substitution method for estimating TSR was first described by Perry (1951) in rats and Henneman, Griffin and Reineke (1952) in sheep. A modified method has been developed for rats (Reineke and Singh, 1955) and several other species (Reineke, 1959). It had great advantage over the goiter prevention method in that it used fewer animals and it was the first method based on isotopes of iodine. The method involves three important points: 1) the thyroid will rapidly accumulate "trap" a large amount of exogenous iodine, 2) exogenous thyroxine will block release of thyroxine from the thyroid in proportion to the dose of thyroxine given, and 3) labeled iodine is

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converted to thyroxine exactly as unlabeled iodine is converted. Following injection of ^{131}I, exogenous thyroxine is administered at regular intervals until the point is reached when no more labeled hormone is released from the thyroid (i.e., when ^{131}I from the thyroid is completely inhibited). That amount is believed to be the daily thyroid secretion rate.

The direct output method for TSR was first designed by Reineke (1964). The method is free from the use of goitrogens and exogenous thyroxine. It involves the measurement of thyroidal ^{131}I turnover and thyroidal iodine content. When the product obtained from multiplying these two parameters was multiplied by a correction factor for the different activities of T_4 and T_3, an estimate of daily TSR was computable. In practice external counts of the thyroid were taken and were expressed as percent injected ^{131}I dose and plotted against time on semi-log paper. Thyroidal ^{131}I output constants were calculated as follows:

$$X = \frac{0.693}{t_{\frac{1}{2}} \text{ (days)}} \qquad (1)$$

$$K'4 = 1-e^{-X} \qquad (2)$$

$$K4 = \frac{K'4}{1-(U/100)} \qquad (3)$$

where t = 1 day, e = base of natural logarithms, U = percent of thyroidal ^{131}I uptake extrapolated to zero time, K_4 = fractional ^{131}I output rate per day corrected for recycling

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of metabolized ^{131}I (Reineke and Lorscheider, 1967). The output rate constant is multiplied by the amount of thyroidal ^{131}I to obtain the secretion rate.

Reineke (1964) and Singh et al. (1968) compared the thyroxine substitution method with the direct output method for evaluation of TSR. In domestic fowl the direct daily output of thyroxine (1.2 µg t_4/100 gm b.w. daily) is lower than in the thyroxine substitution method (2.0 µg t_4/100 gm b.w. daily). The goiter prevention method was also found to be higher than the other two methods (2.32 µg t_4/ 100 gm b.w. daily). When examining the merits and demerits of each method the authors were slightly more inclined to believe the direct output method which they felt did not overestimate TSR as the other methods had.

Thyroidal uptake and release of ^{131}I has also been used as a parameter for measuring thyroid function and although it is not used to quantitatively measure TSR it is a useful tool for clinical and qualitative recognition of thyroid malfunction. Flamboe and Reineke (1959) found no relationship (in goats) between TSR and either ^{131}I % uptake or ^{131}I output rate. Hoersch, Henderson, Reineke and Henneman (1961), using sheep, confirmed that because of a low negative correlation between TSR and zero time % uptake that ^{131}I is not a reliable quantitative estimation of T_4 production.

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Clinically, Greenberg (1966) found that primary and secondary hypothyroidism in humans can be differentiated using ^{131}I release rate. Goyings et al. (1962) used 24 hour ^{131}I uptake as a method for diagnosing hypothyroidism in dogs.

The thyroxine degradation method for TSR evaluation is predicated upon the validity of the assumption that the rate of thyroid hormone degradation is equal to its rate of secretion. In this method a known small amount of labeled thyroid hormone is injected intravenously and blood samples are withdrawn at regular intervals. The nonmetabolized radioactive thyroxine is measured in the serum or plasma. Biological half life, fractional turnover rate, thyroxine distribution space and total extrathyroidal thyroxine may also be determined from the thyroxine degradation data. From these data an estimate of the amount of T_4 secreted daily can be made.

TSR's have been determined in numerous species using the thyroxine degradation method. The first in sheep was by Freinkel and Lewis (1957). Post and Mixner (1961) used it on dairy cattle. It was used in man by Sterling et al. (1954), Ingbar and Freinkel (1955), Sterling and Chodos (1956) and Gregerman et al. (1962). Oddie et al. (1966) reviewed the available literature on human TSR by TDR. They pointed out that thyroxine distribution space (TDS) did not change with height or age but did increase with

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increased weight. TSR was lowered with aging and also decreased in hypothyroidism and hypometabolism. TSR did not change with weight or height in man.

Gregerman in 1963 found that in rats the TDR-TSR was higher in the female than the male during cold exposure. TDR-TSR was also found to increase in old rats along with TDS. Singh, Reineke and Ringer (1968) and Singh (1966) found that TDR-TSR for normal White Leghorn chickens was 2.03 µg/100 g/day and averaged 1.59 and 1.02 in 56 week old chicks and goitrogen-treated chicks respectively. They also found that the TDS values for the 7-week-old chick was significantly higher than in the 56-week-old chicken. Gregerman (1962) found that in euthyroid men thyroxine degradation and TDS decreased with age at about the same rate as basal metabolism (BMR)

Examination of thyroid function by Reineke and Singh (1955) using the thyroxine substitution method indicated that TSR for adult female rats was 2.21-2.56 µg/100 g b.w./ day. Lorscheider and Reineke (1972) using the "direct output" method found that TSR was significantly reduced during lactation or when dietary iodine is low. Beltz and Reineke (1968) by the direct output method pointed out that neonatal rats have a very low TSR. At weights of approximately 22 gm they begin reaching adult TSR levels. Pipes et al. (1963) reported that TSR in beef cattle was 0.27 $\pm$ 0.006 mg/100 lb b.w. which was lower than in dairy cattle

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(0.40 ± 0.013). Romack et al. (1964), using the replacement or substitution technique in swine found TSR to average 0.39 mg/100 lb b.w. Flamboe and Reineke (1959) using the thyroxine substitution method in goats found that TSR ranged between a high in October of 0.336 mg/100 lb b.w. to a low in July of 0.178 mg/100 lb b.w.

In mink, using the thyroxine substitution method, Reineke et al. (1960) reported that mink TSR was 0.95 µg/100 gm b.w.

Thyroxine Binding Proteins

It is generally agreed that circulating thyroxine exists almost entirely in a dissociable complex with plasma proteins. Gordon et al. (1952) were first to describe thyroxine associated with an alpha-globulin in plasma which was later termed thyroxine binding globulin (TBG). Numerous investigations have described the TBG as having a high affinity but occurring in low concentrations. Ingbar (1958) described another plasma entity (albumin) with a capacity to bind thyroxine. Albumin was found to be a thyroxine binder but its characteristics were opposite those of TBG. It had an extremely low affinity for thyroxine, but had a high serum concentration. A third substance, pre-albumin, was also discovered to have an affinity for thyroxine. It was, therefore, assigned the name thyroxine-binding prealbumin (TBPA) (Robbins and Rall, 1960; Ingbar, 1963; Woeber and Ingbar, 1968). Thyroxine binding prealbumin has

an affinity for T_4 ranked between TBG and albumin. Thyroxine binding globulin is the most important thyroxine carrier and TBPA is recorded as second in line of importance. Serum albumin in many species has very little function as a T_4 carrier; deer are a notable exception, because of very high serum T_4 (Byrne, Reineke, Ullrey and Youatt, 1974). In man and most other mammals 60-70% of the T_4 is carried by TBG, 30-40% by prealbumin and less than 10% by albumins. Zaninovich et al. (1966) discerned that thyroxine is the primary hormone bound to thyroxine binding globulin, whereas, triiodothyronine is not significantly bound to TBG.

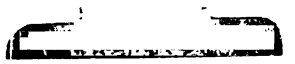
Almost all of the circulating thyroxine is bound to carriers and only about 0.05% is present in the free form (Robbins and Rall, 1960). It is generally agreed that only the free form is able to affect the tissues. Hillier (1970, 1971) reports that the dissociation rate of thyroxine from TBG to free T_4 in solution has a biological halftime ($t_{\frac{1}{2}}$) of 38.6 $\pm$ 2.1 sec. at 37°C. Binding of thyroxine to TBG according to Hillier occurs "loosely" within 0.1 sec. but to complete the normal binding some 20 seconds elapse. The $t_{\frac{1}{2}}$ for thyroxine dissociation from thyroxine binding prealbumin is 7.9 sec. at 37°C which is much faster than release from TBG. Binding of T_4 is "looser" with TBPA than with TBG and the carrying capacity is much lower. Dissociation as thyroxine from TBG and TBPA has its own separate

exponential decline which suggests that each has characteristically different types of binding sites each with different affinities.

Effects of Temperature, pH and Dilution on Thyroxine Binding Proteins

Temperature greatly affects the ability of thyroxine binding proteins to carry thyroxine. Both quantity and rate of dissociation are affected by temperature. Hillier (1971) found that $t_{\frac{1}{2}}$ for thyroxine dissociation from TBG was 38.6 seconds at 37°C but was 8.1 minutes at room temperature (25°C), a 12 fold increase. The $t_{\frac{1}{2}}$ for thyroxine dissociation from thyroxine-binding prealbumin was 7.9 sec. at 37°C but was 53 seconds at 25°C which equals more than a 7 fold increase in rate of dissociation. At 31°C (pH 7.4) prealbumin binding was 30% greater than at 37°C (Lutz and Gregerman, 1969). Etta (1971) also found that a temperature optimum of 37°C was necessary to restrict binding of thyroxine to TBG in several species including man.

The effect of pH has been examined *in vitro* using several buffering systems and is thought to play a role in T_4 transfer to the tissues. It is generally agreed that the buffering system employed is as important in effecting thyroxine binding specificity as the pH. Summarizing the work of several authors who used barbital buffering systems of pH 8.6 and above, they reported inhibition of



binding to albumins and prealbumins but no reduction in binding to globulins (Robbins and Rall, 1960; Lutz and Gregerman, 1969; Braverman et al. 1967; Keane et al., 1969; and Coutsoftides and Gordon, 1970). Binding to albumin was maximal at pH 8.6 when sodium phosphate buffer was used (Antoniades, 1960). Barbital buffer had the quality of interfering with the albumin and prealbumin binding sites at pH 8.6 and was therefore uniquely suited for use in quantifying TBG capacities.

High dilution of thyroxine decreases binding to albumins in blood and serum in vitro but has little effect upon binding to globulins. Murphy and Pattee (1964) reported that a dilution of 1:32 was sufficient to reduce the feeble albumin binding to almost nothing. Murphy and Pattee (1964) and Etta (1971) have shown that a dilution factor of above 1:32 plus barbital at pH 8.6 will effectively inhibit binding to albumins and prealbumins but have no significant effect upon TBG binding.

Effects of Estrogen and Sexual Maturity on Thyroxine Binding Proteins

Sex-related differences in thyroxine binding to its serum receptors is often a function of other non-thyroidal hormones. Dowling et al. (1956) demonstrated that pharmacological doses of estrogenic hormones increase the T_4-binding capacity to TBG. Pharmacological doses of androgenic or anabolic hormones, however, decrease the binding

capacity of TBG. It has been demonstrated by Braverman et al. (1967) that sex related differences do naturally occur in humans. Females were described as having a higher T_4 binding capacity for TBG and a lower capacity for T_4-binding prealbumin than males. Hyperestrogenicity either due to exogenous administration of estrogen (Dowling et al., 1956a, Zaninovich et al., 1966) or due to increased engogenous levels as in gestation (Dowling, 1956b; Musa et al., 1969) is followed by an increase in TBG-capacity. Estrogen affects other factors in thyroxine regulation. It enhances iodine trapping (T/S) and radioiodine uptake by intact, hypophysectomized, or gonadectomized rats without necessarily altering release of thyroxine. Furthermore, TSH secretion is enhanced by low doses of estradiol but is inhibited by high doses (Fisher and D'Angelo, 1971).

Puberty effects a marked change upon TBG capacity. TBG was shown by Riecansky (1967) to be independent of sex and age factors in prepubertal children. Females, because they entered puberty much sooner than boys, showed a higher TBG-binding capacity at age 15 than boys. The sex steroids therefore modify the binding behavior of the thyroxine binding proteins.

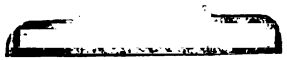
Thyroid Hormone and the Reproductive Cycle

It is well-established that severe hyperthyroidism or hypothyroidism is detrimental to reproductive performance and will often precipitate aberrations in the estrous cycle

(Reineke and Soliman, 1953). The causes of thyroid-induced reproductive disfunction are at least four fold: 1) T_4 affects primary sex tissues, 2) effect of thyroid hormones upon the central nervous system and gonadotropins, 3) effect of steroids upon thyroxine concentration, and 4) thyroxine-induced changes in metabolism or a breakdown of reproductive steroids. Experimental evidence for these mechanisms has been completely reviewed through 1952 by Reineke and Soliman (1953). Marked species differences in reproductive response to hypothyroidism and hyperthyroidism also exist (Nalbandov, 1964).

Hypothyroidism affects secondary sex characteristics as early as puberty. Vaginal cornification and ovulation in rats occurs on the day of vaginal opening (34 days of age). Thyroidectomized rats, however, experienced vaginal cornification and ovulation much later than the day of vaginal opening (Hagino, 1971). Anesthetization with pentobarbital followed by an injection of PMS (pregnant mare serum) caused ovulation at a normal age in thyroidectomized pubertal rats. Hagino concluded that continuous exposure of the central nervous system to thyroid hormone is necessary for regulation of gonadotropin secretion.

Schultze and Noonan (1970) found that exogenous thyroxine increased uterine metabolism at the proestrus stage but decreased estrogen-induced high uterine metabolic rate. They also described an enhanced ovulation rate, implantation



rate and litter size with a thirty microgram dose of L-thyroxine 6 days prior to breeding and discontinued on the day of breeding. Soliman and Reineke (1954) demonstrated that ^{131}I uptake by the thyroid gland of mature mice varied with the estrous cycle and that thyroids attained maximal ^{131}I uptake during proestrous.

The effect of thyroid activity upon delayed implantation of blastocysts in rats was studied by Holland et al. (1967). Their technique for inducing delayed implantation was to ovariectomize rats on the third day after mating and maintain them on progesterone during the delay period. Implantation was subsequently induced by the administration of 1 µg of estrogen daily following the 9th day after mating. Upon autopsy on day 14, hyperthyroid rats possessed more implantation sites than controls; however, hypothyroid rats had fewer implantation sites than the controls. Their results suggested that a moderate level of hyperthyroidism is beneficial to implantation while hypothyroidism tends to be detrimental to blastocyst implantation.

Non-pregnant rats show the highest thyroid gland activity, which occurs during pro-estrous in association with an increase in pituitary TSH. This increase like the increase in thyroid gland activity does not occur when ovulation is blocked (Newcomer and Brown-Grant, 1971). TSH is also observed to increase in early pregnancy.

Schreiber et al. (1967) pointed out that thyroid hormones play a decisive role in regulating hypertrophy and metabolic activity of the anterior pituitary under various conditions. Thyroxine administration was observed to prevent estrogen-induced anterior pituitary hypertrophy, but none of the other actions associated with estrogen were observed to be altered. Their group also found that ^{125}I-thyroxine binding to pituitary proteins in vitro was increased under estrogen administration. Furthermore, large doses of thyroxine not only blocked anterior pituitary hypertrophy but increased in vitro ^{125}I-thyroxine binding to pituitary proteins. Testosterone was reported to stimulate the same thyroxine phenomena as estrogen in the rat (Schreiber et al., 1967).

Association of thyroxine with anterior pituitary mitochondria was observed by Schreiber et al. (1971). Anterior pituitaries were incubated with labeled thyroxine first, then by centrifugation the following 4 fractions were separated: nuclear (2,000 g), mitochondrial (20,000 g), microsomal (105,000 g), and cytosol. Counting of the 4 fractions indicated the preferential association of thyroxine with the mitochondria. Significantly higher amounts of ^{125}I-thyroxine were found in the mitochondrial fractions from animals receiving estrogen treatment than those without exogenous estrogen. This association of thyroxine for the anterior pituitary mitochondria is exceptional compared with

other tissues where the attraction was not observed (liver, kidneys, brain, heart).

Thyroid Hormone and Pregnancy

Pregnancy is accompanied by important modifications in thyroid function. Hypertrophy and hyperplasia are observed first in early pregnancy and many investigators have considered the thyroid goitrous during pregnancy. In addition to these anatomical changes the following physiological parameters are also increased: thyroid secretion rate, thyroxine binding globulin, protein bound iodine, thyroid iodide uptake, iodine pool and butanol extractable iodine (Heineman et al., 1948; Man et al., 1969; Robbins and Nelson, 1958). Absolute free thyroxine and thyroxine turnover rate were not altered by gestation (Ingbar et al., 1965; Dowling et al., 1967). Plasma TSH levels are found to increase at the sixth week of gestation in humans and remain higher than normal until after parturition when they return to normal (0.24 mU/ml normal and 0.48 mU/ml during pregnancy) (Lemarchard-Beraud and Mean, 1970). It was also found that TSH of slightly hypothyroid pregnant women is lower than in controls both before and after parturition. Pregnant rats and controls were found to have nearly identical thyroid ^{131}I regression slopes but uptake rates were slightly but consistently lower in pregnant rats (Iino and Greer, 1961).

In contrast to the human and rat the golden hamster has reduced PBI and thyroidal accumulation of ^{131}I during the last half of pregnancy (Galton, 1968). Urinary excretion of ^{131}I-T_4 was greatly increased when examined during the late pregnancy in the hamster. The increased excretion of iodide from T_4 was associated with a fall in the concentration of serum T_4. After delivery, urinary iodide excretion and serum T_4 concentration returned to normal. Galton (1968) studied T_4 metabolism in vitro and found that deiodination in liver of 20 day pregnant hamsters was normal, however, it was very high in comparable preparations of fetal tissue. The rat pregnancy was associated with an increased fractional turnover and probably an increased absolute turnover of T_4 in the tissues. The increase in rat serum is probably due in part to the increase in thyroxine binding globulin in the serum and may also be attributed in part to normal deiodination in fetal tissue.

Hernandez, Etta, Reineke, Oxender and Hafs (1972) reported that fetal T_4 in cattle serum was approximately twice maternal levels between 90 and 180 days. TBG was completely saturated in fetal serum but only 2/3 saturated in maternal serum. Information concerning transport of thyroxine across the placenta is often contradictory and is probably dependent upon species. Some species may derive fetal thyroxine crossing the placental barrier while others have extremely active fetal thyroids which produce a higher

circulating level of thyroxine than the mother.

Human and rabbits during the last trimester and particularly near term show significantly larger maternal to fetal transplacental differences in thyroxine (Fisher et al., 1964). Mice, sheep and bovine feti (Waterman, 1958; Hernandez et al., 1972) have very high serum thyroxine levels and anatomically the fetal thyroids appear large and much more active than maternal thyroids (Fisher et al., 1964; Fisher and Lam, 1974).

Hotelling and Sherwood (1971) found that circulating triiodothyronine (T_3) in humans increases from 196 ng/100 ml during the first trimester to 299 ng/100 ml in the third trimester. The increase in T_3 parallels that of T_4 and TBG. They also pointed out that T_3 in vivo is significantly bound to TBG. At birth umbilical levels of T_3 are only 193 ng/ml or approximately equal to the first trimester maternal levels.

Thyroid Hormone and Lactation

Circulating thyroxine levels of the young neonate seems to be a function of the neonates ability to mimic adult locomotion and feeding behavior. Ungulates and guinea pigs seem to be quite active when born and their serum thyroxine levels are also elevated above maternal levels for some time after parturition. Pals, Reineke and Shaw (1973) showed that in young guinea pigs (Cavia porcellus) which is

one of the most precocious neonates to have serum thyroxine levels rise 4 times the prenatal level within 1 hour after birth. Thyroxine level declined exponentally at the rate of 2.3% per hour. They reached adult T_4 levels at 3-6 weeks of age at which time they were almost adult in size.

Hernandez et al. (1972), reported that serum thyroxine levels of bovine calves at birth were about twice adult levels and that adult values were approached at the end of 6 days. TBG also declined following birth, releasing more T_4 for metabolic purposes. It was suggested that the days of high thyroxine levels following birth were necessary for the adjustment of the newborn calf to its new environment.

Maternal reaction to the lactating offspring is often quite severe. Some cattle have even become severely hypothyroid and in areas of low iodine such as the goiter belt in the Northern Midwest and Pacific Northwest have even died from the lack of replacement of iodine removed by the suckling calf. Hernandez et al. (1972) found that during early lactation maternal T_4 is approximately one-half neonatal levels. Lorscheider and Reineke (1972) reported that during heavy lactation in rats thyroidal iodine supplies were depleted, but that accumulation became more efficient. Flamboe and Reineke (1959) noted substantial losses of iodine in the milk of goats. They suggested that under iodine deficient conditions a functional thyroid deficiency

could occur during lactation.

Indications are that thyroxine is actively secreted into milk. Flamboe and Reineke (1959) and Reineke (1961) found that administered ^{131}I was secreted into milk. The phenomenon has been seen in numerous other species (see Reineke, 1961). About 92% of the ^{131}I found in goats milk was found as iodide and the remaining 8% was protein bound. Reineke (1963) reports that under conditions of lactation mammae compete with the thyroid for available iodine, resulting in reduced thyroxine formation. He found that the major component of iodine secreted into milk was monoiodotyrosine (52%). It is assumed that much more of the ingested iodinated proteins reach the neonate than would reach an adult since gastric acids of newborn are not in abundance during lactation particularly in carnivorous animals.

It has been found by Byrne et al. (1974) that deer lactation is similar to cattle. The extremely high T_4 at birth (26 µg%) drops to almost adult levels during the sixty plus days of lactation.

STATEMENT OF THE PROBLEM

It is well-known that mink are one of the most sensitive mammalian species to many toxins such as diethylstilbestrol, DDT, DDE, dieldrin and other pesticides. PCB's chemically resemble these compounds. Mink are therefore excellent models for describing the mechanisms of action of these substances and might point the way of possible harm to man.

Mink reproduction has been found to be severely reduced by PCB's; however, the physiological mechanisms were not known.

Hypo- and hyperthyroidism have been known for years to cause reproductive failure in many species and it is known that PCB's have induced weight and morphological changes in the thyroid gland of lower animals.

It was thought that PCB's might alter the balance of hormones necessary for normal reproduction and that since PCB's have been seen to alter thyroid anatomy, changes in production and circulation of thyroid hormone might be associated with PCB's.

MATERIALS AND METHODS

I. Animal Treatment Protocol

A. Animal Care and Reproductive Cycle

All mink in this study were housed and cared for at the Michigan State University Fur Animal Project Ranch on the south campus of Michigan State University.

The estrous cycle of mink begins with estrus on about March 1st and extends to March 15th. Estrus is followed by a period of delayed implantation which occurs between March 1st and April 1st. Implantation on April 1st is followed by 5 weeks of gestation up to May 8th. Lactation continues from approximately May 8th to June 1st. Weaning takes place around June 1st. Diestrus extends throughout the year until pre-estrus in late February.

B. Experiment I

Thirty-two adult female mink (Mustella vison) were randomly assigned to four groups of eight. All animals were fed a high protein balanced diet, ad libitum (Appendix B). The groups received 3 concentrations of polychlorinated biphenyl (PCB) (Aroclor®1254[1]; Monsanto Chemical Co., St. Louis, Mo.) added to their normal daily

[1]® Registered trademark for polychlorinated biphenyl compounds, Monsanto Chemical Co., St. Louis, Missouri.

diet (5 ppm, 2 ppm, 0.5 ppm) and a control.

Blood samples and body weights were taken at 28-day intervals from September 19, 1973, through June 26, 1974. Special diets were initiated on September 21, 1973, two days after the first blood sampling, and were continued through the last sampling date in June. Plasma was separated from the blood and analyzed for circulating thyroxine (see Serum Thyroxine Determination).

C. Experiment II

Sixteen adult female mink were randomly assigned to two groups of eight. Each was fed as described in Experiment I, except that Group I received 5 ppm (Aroclor 1254) PCB. The PCB diet was begun in late October and continued throughout the course of the experiment. On February 19th, when the animals were pre-estrus, a sample of blood was collected by toe clipping for determination of plasma thyroxine level and circulating thyroxine binding globulin (TBG) capacity. Three days later on February 22, 1974, thyroxine secretion rate (TSR) experiments were performed on the mink by the thyroxine degradation method. These same animals were again bled for a TSR experiment on April 12th, during the gestation period of mink, and again on May 15th during lactation. In each instance blood for T_4 analyses was withdrawn 3 days prior to the TSR experiment and body weights were recorded to $\pm$ 5.0 gram. An additional

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group of five mink were added to experiment II in January, 1974, to be used to assess the TSR procedure in mink. Two lactating controls were also added to the May 15th TSR experiment. One week following the last TSR experiment, on May 21st, the original group of 16 females plus two extra lactating controls were killed and autopsied. Body weights were recorded to the nearest 5.0 g. A cursory visual examination was also made of the heart, lungs, stomach, and intestines. The thyroids were placed in glass vials containing Dietrich Fixative and were imbedded in paraffin the following day. They were later sectioned at 6 microns and stained with hematoxylin and eosin.

D. Experiment III

This experiment was designed to delineate the difference in thyroxine level between female and male mink. Blood samples were drawn by the toe clipping from mink of both sexes at irregular intervals between autumn 1972 through summer 1973. Plasma was stored at -20°C until ready for thyroxine analysis.

E. Experiment IV

Five female mink which had been maintained on a normal ranch diet, were killed in late December during late diestrus. Sufficient blood was collected to supply that needed to run a thyroxine binding curve (see Appendix C for the method). Plasma samples from the animals used for the

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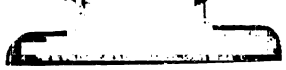
TSR study in experiment II were used to assess the T_4 binding capacity of the circulating thyroxine binding globulin (TBG) and the saturation index.

II. Serum Thyroxine (T_4) Determination

Serum thyroxine levels were measured by the Tetrasorb-125 method (Abbott Laboratories, Radio-Pharmaceutical Division). The method is a competitive protein binding assay specific for total serum or plasma L-thyroxine. Briefly, the principle of the method is that thyroxine extracted from plasma competes with tracer ^{125}I-thyroxine for binding sites in a given quantity of thyroxine binding globulin (TBG). Levels of "cold" endogenous thyroxine will compete with labeled T_4 for the TBG sites with the assumption that both have equal affinity for the available sites. When equilibrium is reached, the addition of a resin-impregnated sponge will bind the unbound thyroxine but not the TBG-bound thyroxine. This renders the two components separable. Therefore, as cold T_4 or plasma T_4 increases it displaces additional labeled T_4 from the TBG which is then absorbed by resin sponge. The TBG-bound thyroxine is washed from the sponge and the ratio of the radioactive counts within the resin sponge to the initial radioactive counts is proportional to the unlabeled thyroxine.

Blood samples of approximately 2 ml were obtained by the toe clip method. Plasma was separated from the formed



elements within 4 hours after drawing the samples.

III. Thyroxine Secretion: Degradation Rate

A. Solutions

^{131}I-L-thyroxine in 50% aqueous propylene glycol (Amersham/Searle Radiopharmaceuticals, Arlington Heights, Illinois), because of its short half-life of 8 days, was purchased fresh for each of 4 TSR experiments. It was diluted in glass-distilled water so that a 1 ml injection would administer a dose of approximately 45 µCi. Although most of the glassware had been siliconized, 0.25% bovine serum albumin (Fraction V) was added as a carrier to prevent thyroxine adhesion to the glassware.

Standards were prepared such that a 45 µCi/ml dose was diluted 200, 250, 300, 350 and 400 times to approximate the dilution which a dose of 45 µCi/ml would undergo in an adult female mink of approximately 1000 grams.

B. Injection Procedure

Thirty minutes before each injected dose of ^{131}I-L-T_4 was aministered, a 1 ml subcutaneous injection of sodium thiocyanate at 50 mg/ml was administered to each animal in order to block the iodine trap of the thyroid, thereby preventing recycling of labeled T_4.

To anesthetize the animals 0.2 ml C-I 744 (Parke Davis, Ann Arbor, Michigan) was injected intramuscularly (IM).

Each animal was unconscious for approximately 10 minutes and another 15 minutes elapsed for complete recovery. Hair was removed and a small incision was made in the neck. ^{131}I-thyroxine solution was then injected intravenously (IV) into the exposed jugular. Extreme caution was taken to assure that all of the labeled thyroxine was injected directly into the vein. Sutures were then applied to muscle and dermal layers. The thick hair covering all superficial veins made the minor operation necessary for IV injection.

After each animal had become active following anesthesia each was taken back to its individual cage within the colony to prevent any additional stress. After a four-hour distribution time, blood samples were taken at two-hour ($\pm$ 1 min.) intervals up to 12 hours.

Plasma samples were then frozen and stored at -4°C until used. Because mink have very high hemotocrits (approximately 60%) only about 0.8 to 0.9 ml of plasma was obtained at each sampling period.

Procedure for T_4 analysis was modified from the method found in the Abbott Laboratory Tebrasorb Manual. Plasma volumes of 0.6 ml were used instead of 0.3 ml to compensate for the comparatively low T_4 level in mink. A standard curve was made from a working standard solution of L-thyroxine that was purified in our laboratory. Standards contained 2.5, 5 and 10 mg/100 ml plasma. Uncorrected thyroxine values

were obtained from the slope and intercept of the standards and corrected T_4 values were obtained by dividing the uncorrected values by 0.79 which is the extraction efficiency of 95% ethanol.

C. Sample Collection

At each sampling period a toe was clipped and approximately 1 ml of blood was collected in a heparinized paraffin cup, transferred to a polypropylene microcentrifuge tube and plasma was separated by centrifugation in a Beckman 152 microcentrifuge. Each animal was then returned to its cage. A 20 microliter aliquot of plasma was transferred to a 1.3 cm x 8.6 cm polypropylene tube and set aside for counting. All tubes were then held at room temperature until all samples had been drawn and were summarily counted along with standards for one minute in a gamma well counter, model DS-5 (Nuclear Chicago), with a scaler-analyzer (Nuclear Chicago, Des Plains, Ill.) set to count at the ^{131}I peak.

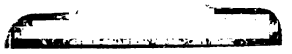
D. Computations

Computation of percent dose per ml plasma

a. Counts per microliter were converted to counts/ml as follows: CPM-bkg/20 µl plasma x 50 = CPM-bkg/ml

b. Injected dose = µCi experimental dose (e.g., 45 µCi in standard)

c. Injected count = (standard cpm-background) x (injected dose)



d. Percent ^{131}I-L-T_4 dose/ml serum =

$$\frac{\text{Counts-background/ml plasma (a)}}{\text{Injected count (c)}}$$

Percent dose/ml serum was then calculated for each of 16 animals at intervals of 4, 6, 8, 10, and 12 hours after injection. The percent ^{131}I dose per 100 ml serum was regressed against time by the equation:

$$\log y = a + bx$$

where log y = percent dose, x = time.
a = the log intercept at injection time.
b = the slope of the regression line.

Computation of Degradation Rate Constant

Data were converted to the log n form by the equation:

$$y = e^{-xt} \qquad \text{I.}$$

where x = slope, e = the base of the natural logarithm.
y = percent injected dose/ml plasma at time t.
(The quantity of e^x is most easily obtained from a table of descending exponentials.*)

$$(1-e^{-x})\ (2.302) = \text{Degradation Rate Constant/hr.} \qquad \text{II.}$$

where 2.302 = factor used to transform $\log_{10}$ to natural logarithms.

Computation of Biological half-life $t_{\frac{1}{2}}$

$$t_{\frac{1}{2}} = \frac{0.301}{\text{slope}}\ (b) \quad \text{(the numerical slope, not the algebraic } \log_{10} \text{ slope)} = \text{hours} \qquad \text{III.}$$

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*Tables of the Exponential Function e^x National Bureau of Standards, Applied Mathematics Series 14, 4th ed., U. S. Government Printing Office, Washington 25, D. C., 1961.

where:

$t_{\frac{1}{2}}$ is defined at the amount of time in hours required to degrade half of the ^{131}I-L-thyroxine concentration present at time zero. The theoretical percent of ^{131}I-L-T_4 present at time zero is obtained by extrapolating back from the data points or was commonly computed from the intercept of the linear regression line.

Computation of thyroxine distribution space

TDS/mls = 100% dose at time zero IV.

or TDS/ml - 100/Anti-Log_{10} intercept

TDS/100 gm body weight = TDS/gm body weight/100 V.

Computation of Extra Thyroidal Thyroxine

Ett = (TDS/100 gm body weight) (T_4 µg/ml serum) = µg T_4 IV.

Computation of Thyroxine Secretion Rate

TSR = (Ett) (Rate constant K) (24 hrs.) = TSR/100 gm/day IIV.

For TSR computations in this manner at least 4 assumptions must be made. First, secretion rate must equal degradation rate. Second, ^{131}I-thyroxine must be distributed to all the pools. Third, ^{131}I-T_4 is degraded just as non-labeled thyroxine is degraded. Fourth, the NaSCN has no peripheral effect on iodine metabolism as it does in the thyroid.

IV. Thyroxine Binding Globulin (TBG) Capacity and Saturation Index

Samples were first analyzed for serum thyroxine and the remaining serum was frozen for later use. Briefly the method, modified from a method by Etta (1971), involves first a determination of the level of cold hormone which is required to saturate and "compete out" the ^{125}I labeled thyroxine. The total amount of cold hormone required to saturate the TBG is the sum of the endogenous T_4 plus the exogenous T_4, both labeled and cold.

A thyroxine binding curve was made for the TBG capacity at varying thyroxine concentrations. On the ordinate was "percent T_4 bound" and on the abscissa was "T_4 used µg percent (exogenous and endogenous)". From the plateau of the binding curve, the concentration of total T_4 necessary to saturate or exceed the TBG-capacity at 37° was observed (see Appendix C for a detailed description of this method). This concentration was used to determine the binding capacities of the TBG (see Appendix C for procedure and calculations) of mink at selected times under conditions of 5 ppm PCB 1254 and control samples.

Three critical factors are necessary for the TBG *in vitro* to specifically bind thyroxine. They are: 1) the barbital buffering system at pH 8.6 completely inhibits binding to albumins and prealbumins, 2) by maintaining a high dilution factor of 30-35, binding to albumin which is

usually weak is reduced to null, and 3) incubation at 37° during equilibration of the binding system maximizes binding of thyroxine to TBG and minimizes binding to all other proteins.

The determination of saturation index is simply the serum T_4 divided by the TBG-capacity.

V. Statistical Analysis

Data for the experiments was statistically analyzed using split plot factorial designs with block treatment. Analysis of several sources of variance was undertaken using a program adapted from one written by Edward Cogger. The main IBM 6500 computer at the Michigan State University Computer Center was used to handle the ANOVA analyses.

Hartley's F max test was used to assess the homogeniety of the variances. Scheffé's F test was used to separate mean differences. All P values reported herein are at the 0.05 level of confidence. See Appendix H for the equations of the Scheffé F test.

Coefficient of correlation was used to test the strength of the linear relationship in the thyroxine degradation regression line.

RESULTS

Experiment I--Plasma Thyroxine Level Changes with Three Levels of PCB

Throughout the course of this nine-month study significant differences were found between the PCB treatment effects upon T_4 levels and also between the time effects (B) (see Statistics Table 1). Interactions between the two factors (AB interactions) were also significant. The observed differences in T_4 levels (see Figure 1) at various time periods were, therefore, often the result of both differences in PCB concentration and annual cycles. The appearance of the lines meandering in Figure 1 may be a function of the changes associated with the estrous cycle, reproduction, gestation and lactation interacting with those of the PCB treatments.

Thyroxine levels of all four groups were approximately equal to 2.10 µg/100 ml at the initiation of the experiment prior to PCB feeding. All four groups increased significantly through September; however, the controls and 5 ppm PCB groups were significantly higher than the remaining two groups in October (see Table 2 for mean comparisons using Scheffé F test). At 3.26 µg percent the controls were at their highest measured levels in October and the difference

Table 1. Factorial Analysis of Variance Table of Data in Experiment I

	Source	SS	df	MS
1	Between subjects	18.3828	31	1.4141
2	A (type of signal)	9.84539	3	9.8453
3	Subj. w. groups	8.53742	28	0.71145
4	Within subjects	347.2331	288	12.4012
5	B	242.803	9	21.4015
6	AB interaction	34.6132	27	17.3066
7	B x subj. w. groups	69.8165	252	2.9090
8	Total	365.6159	320	8.9175

F_A = 10.763234*/2.95

F_B = 97.37664*/1.88

F_{AB}= 4.62724*/1.48

*P < 0.05

Table 2.

Treatment
Jan. Sp
Jan. Sp
Jan. Sp
Jan. Co
Jan. Co
Jan. Sp
Jan. Sp
Jan. Sp
Feb. 2
March C
April C
May C
May C
May C
June C

MS Subj

P<0.05

Table 2. Statistical Comparison of Data in Experiment I Using the Scheffé F Test

Treatment	Comparison (A Effect)	F Test Statistics/Critical Value	
Oct.	5ppm + control vs. 2 ppm + 0.5ppm	31.33 / 10.02	
Nov.	2ppm vs. control + 0.5ppm	8.43 / 6.68	
Nov.	5ppm vs. 0.5ppm	60.83 / 3.34	
Dec.	Control vs. 5ppm + 2ppm + 0.5ppm	177.6 / 10.02	
Jan.	Control vs. 5ppm + 2ppm + 0.5ppm	32.76 / 10.02	
Jan.	5ppm vs. 0.5ppm	0.165/ 3.34	NS
Jan.	5ppm vs. 2ppm	7.09 / 3.34	NS
Jan.	5ppm vs. control	10.89 / 3.34	
Feb.	2ppm vs. control	36.02 / 3.34	
March	0.5ppm + 2 ppm vs. 5ppm + control	15.15 / 10.02	
April	Control + 0.5ppm vs. 2ppm + 5ppm	13.06 / 10.02	
May	Control + 0.5ppm vs. 2ppm + 5ppm	5.56 / 10.02	NS
May	Control vs. 5ppm	1.49 / 3.35	NS
May	0.5ppm vs. 2ppm	4.45 / 3.34	
June	Control vs. 5ppm + 2ppm + 0.5ppm	26.40 / 10.02	

MS Subj w. group = 0.71145; $V_1 = 2$, $V_2 = 28$

$P < 0.05$

continued

Table 2--continued

Time Comparison (B Effect)

Controls	F Critical Value		5ppm	F Critical Value	
Sept. vs. Oct.	F = 3.29		Sept. vs. Nov.	F = 62.7	
Sept. vs. Nov.	7.05		Nov. vs. Feb.	0.176	NS
Feb. vs. Mar.	4.54		Jan. vs. Feb.	12.65	
Mar. vs. April	17.54		Feb. vs. Mar.	28.21	
Feb. vs. April	34.02		Mar. vs. April+May	1.84	NS
April vs. May	19.87		May vs. June	0.99	NS
May vs. March	0.57	NS	June vs. Feb.	0.99	NS
May vs. June	13.27		June vs. Sept.	31.31	
April vs. June	20.29		May vs. Sept.	3.96	
April vs. Sept.	20.72				
April vs. Oct.	0.44	NS			

2ppm	F Critical Value		0.5ppm	F Critical Value	
Sept. vs. Oct.	F = 11.02		Sept. vs. Oct.	15.74	
Sept. vs. Nov.	2.75		Feb. vs. March	0.324	NS
Nov. vs. Dec.	2.30		Feb. vs. April	0.991	NS
Dec. vs. Feb.	0.32	NS	Feb. vs. May	0.143	NS
Dec. vs. Jan.	3.59		April vs. May	0.38	NS
Jan. vs. April	12.65		April vs. June	12.65	
March vs. April	2.45		May vs. June	8.97	
April vs. June	12.65		Nov. + Dec. + Jan. vs. Feb.	17.49	

MS BX Subj. w. group = 2.909; $V_1 = 9$, $V_2 = 252$

$P < 0.05$ Critical Value = 1.88

Figure 1. Effects of long term ingestion of polychlorinated biphenyl upon circulating thyroxine in female mink. Each point equals the mean plasma thyroxine (µg/100 ml plasma) ± the standard error of the mean of 8 animals.

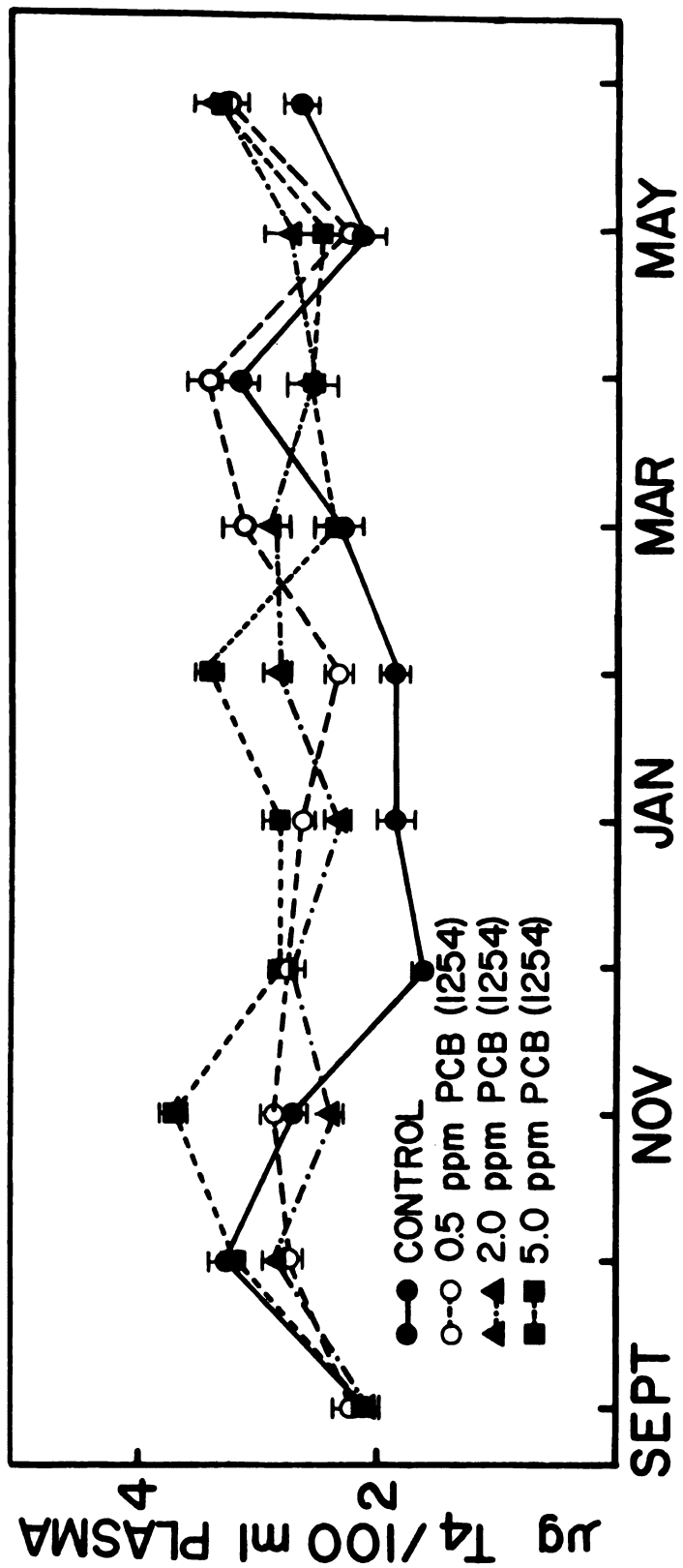

µg T4/100 ml PLASMA
4
2
SEPT
NOV
JAN
MAR
MAY
CONTROL
0.5 ppm PCB (1254)
2.0 ppm PCB (1254)
5.0 ppm PCB (1254)

Figure 1

between October and the second highest control level of 3.12 µg percent during April is significant. T_4 of the 5 ppm PCB group and the controls was almost identical in October. Similarly, the 2 ppm and 0.5 ppm PCB groups were not significantly different in October.

In November the 5 ppm PCB group reached a seasonal high of 3.76 µg percent which is the highest T_4 level of any group and month recorded throughout the duration of the study. The other three groups were significantly lower than the 5 ppm PCB group. Throughout the diestrous period until estrus in March the 5 ppm PCB group remained significantly higher than the control. The controls dropped from their seasonal high in October to their lowest point in December (1.61 µg percent T_4)

During December all three PCB groups were approximately equal at 2.8 µg percent T_4; however, they were significantly higher than the control. Controls were still significantly lower in February at 1.84 µg percent than the other three groups. Controls were similarly reduced in February. February is the only month when a direct dose response relationship is seen between the groups. All four groups are significantly different and are arranged such that the control group is lowest and the 5ppm PCB group is highest. At this period the full effect of the dosages is apparent.

Estrus begins in early March in mink and its influence apparently alters the T_4 levels quite drastically (see

Appendix B for the mink estrous cycle). The 5 ppm PCB group dropped by 30% between February and March to equal the control at 2.3 µg percent. The control increased significantly between February and March from 1.89 to 2.34 µg percent. Groups receiving 0.5 ppm and 2 ppm PCB were significantly higher than the other two groups but were themselves not significantly different. The 0.5 ppm PCB group increased significantly between February and March and reached its annual high (3.47 µg percent) during implantation and early gestation in April.

Between estrus in March through delayed implantation in late March to gestation in early April, the two high PCB groups had significantly lower plasma T_4 concentrations than the control and the 0.5 ppm PCB groups. The wide difference in T_4 at this point exactly coincides with the reproductive performance of these animals (see Reproductive Performance Results, Table 7) where the 0.5 ppm PCB group had the best reproductive performance followed closely by the controls. The two groups on the highest PCB levels had only one kit for 16 females. This one did not survive.

During gestation, between April and May, the T_4 levels in the control and 0.5 ppm PCB groups decreased by 30% and 36% respectively. Decreases such as this are not common during pregnancy in many species. In the 5 ppm and 2 ppm PCB groups thyroxine levels did not change. This would be expected if indeed they were not pregnant.

During lactation, in May, the 3 PCB groups had T_4 levels significantly higher than the control groups. In the 0.5 ppm PCB group T_4 was approximately equal to the 5 ppm and 2 ppm groups. During late lactation, T_4 in the group receiving 0.5 ppm PCB rose to the same level as the other treatment groups. This may be due to a cumulative buildup of PCB's. (See Appendix D for data summarized in Figure 1.)

Experiment II--Estimation of Thyroxine Secretion Rate and Associated Factors

A factorial analysis of variance for each calculated parameter in this study (Table 4) shows that treatment effects (A) were significantly different in all except body weight. In all parameters there were significant differences with time (B). There were significant dose vs. time interactions in TSR, T_4, $t_{½}$, body weight and percent dose at time zero. (See Table 5 for mean comparison using the Scheffé F test.)

In January, 1974 (see Figure 2), when mink were in late diestrus, thyroxine secretion rate of control mink was 0.854 µg/100 g body weight/day. The T_4 was 1.69 µg/100 ml plasma which was not significantly different from the T_4 in control mink at a comparable period in experiment I. The degradation rate constant K equals 0.1172 percent dose $^{131}I\text{-}T_4$/ml plasma per hour. The biological half life ($t_{½}$)

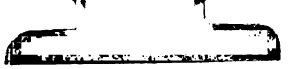


Table 3. Summary of Thyroid Parameters. Means and Standard Errors of the Means from Experiment I. (See Appendix E for Individual Data)

PCB 5 ppm	January	February	April	May	
TDS/100 gm B.W. (ml)		20.04 ±1.72	12.06 ±0.552	27.65 ±2.23	
$T_{\frac{1}{2}}$ (hr)		5.16 ±0.327	4.85 ±0.392	6.49 ±0.35	
K (^{131}I-T_4 hr)		0.1338±0.008	0.1352±0.011	0.0971±0.0092	
T_4 (µg percent)		3.69 ±0.154	2.46 ±0.229	3.12 ±0.223	
Ett (µg T_4)		66.7 ±0.074	29.6 ±2.86	87.2 ±10.9	
TSR (µg/100 gm/day)		2.35 ±0.216	0.909 ±0.001	1.93 ±0.175	
N =		8	7	8	
Control				Lactating	Non-lactating
TDS	18.19 ±3.14	12.68 ±0.580	8.93 ±0.315	28.39 ±2.45	23.64 ±2.65
$T_{\frac{1}{2}}$	6.00 ±0.541	6.17 ±0.298	7.15 ±0.483	7.88 ±0.23	8.74 ±0.489
K	0.1172±0.013	0.1109±0.0051	0.0970±0.0056	0.0867±0.0024	0.0784±0.0044
T_4	1.69 ±0.191	1.28 ±0.151	1.39 ±0.139	2.05 ±0.108	2.29 ±0.149
Ett	31.9 ±7.89	16.46 ±2.32	12.59 ±1.55	60.2 ±7.30	54.2 ±6.70
TSR	0.854 ±0.175	0.440 ±0.065	0.291 ±0.0357	1.25 ±0.167	1.007 ±0.078
N =	5	7	8	5	3

Table 4. Factorial Analysis A Variance Table: PSR-TDR Data, Experiment II

Source	Body Weight SS	df	MS	Percent Dose at Time Zero SS	df	MS
TOTAL	892902.976191	41	21778	3.844116	41	0.0937
Between Subjects	591819.642857	13	45524	.212802	3	0.0163
A Type	125405.357143	1	125405	.163064	1	0.1630
Subject W Groups	466414.285714	12	38867	.049738	12	0.0041
Within Subjects	301083.333334	28	10752	3.631314	28	0.1296
B	51051.190476	2	25525	3.238897	2	0.1109
AB Interaction	120289.285715	2	80144	.221948	2	0.1109
BX Subj. W Group	120742.857143	24	5405	.170469	24	0.0071
FA	1.075485 /	4.75	NS	13.113939* /	4.75	S
FB	4.721757*/	3.40	S	227.999098* /	3.40	S
FAB	11.125633*/	3.40	S	15.623802* /	3.40	S

Source	$T_{\frac{1}{2}}$ SS	df	MS	TDS/100 gm. BW SS	df	MS
TOTAL	86.211840	41	2.1027	2599.246450	41	63.39
Between Subjects	33.193707	13	2.5536	349.723050	13	26.9018
A Type	24.031736	1	24.0317	162.250060	1	162.2300
Subject W Groups	9.161971	12	0.7634	187.472990	12	15.6227
Within Subjects	53.018133	28	0.0189	2249.523400	28	80.3401
B	20.335633	2	10.1678	1839.918700	2	919.9593
AB Interaction	1.658414	2	0.8292	30.333890	2	15.1669
BX Subj. W Group	31.024086	24	1.2926	379.270810	24	15.8029
FA	10.491950*/	4.75	S	8.423616 /	4.75	S
FB	7.865747*/	3.40	S	58.214405* /	3.40	S
FAB	.641468 /	3.40	NS	.959754* /	3.40	S NS

*P < 0.05

continued

Table 4--continued

Source	Slope SS	df	MS	K SS	df	MS
TOTAL	0.700635	41	0.0170	0.040131	41	0.00097
Between Subjects	0.221958	13	0.0170	0.012210	13	0.00093
A Type	0.167948	1	0.1679	0.007681	1	0.007681
Subject W. Groups	0.054010	12	0.0450	0.004529	12	0.00037
Within Subjects	0.478676	28	0.01709	0.027921	28	0.00099
B	0.179899	2	0.00899	0.010292	2	0.00514
AB Interaction	0.040169	2	0.02008	0.000520	2	0.0003
BX Subj. W. Group	0.258608	24	0.01077	0.017109	24	0.0007
FA	12.438155*/4.75			6.783991*/4.75		
FB	8.347723*/3.40			7.218746*/3.40		
FAB	1.863955 /3.40 NS			0.365068 /3.40 NS		

Source	T_4 SS	df	MS	ETT SS	df	MS
TOTAL	39.262048	41	0.9576	4.218997	41	0.1029
Between Subjects	24.038114	13	1.8491	1.682396	13	0.1294
A Type	21.859286	1	21.8592	1.435120	1	1.4351
Subject W. Groups	2.178829	12	0.1816	0.247276	12	0.0206
Within Subjects	15.223933	28	0.5437	2.536601	28	0.0906
B	5.183348	2	2.5917	1.575217	2	0.7876
AB Interaction	5.521386	2	2.7606	0.205575	2	0.10278
BX Subj. W. Group	4.519200	24	0.1883	0.755810	24	0.0315
FA	40.130345*/4.75 S			23.214828*/4.75 S		
FB	13.763536*/3.40 S			25.009738*/3.40 S		
FAB	14.661141*/3.40 S			3.263911 /3.40 NS		

*P < 0.05

continued

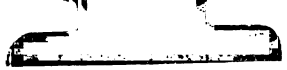


Table 4--continued

Source	TSR SS	df	MS
TOTAL	284.1247057	41	6.9295
Between Subjects	125.1053124	13	9.62384
A Type	120.9432010	1	120.9432
Subject W. Groups	4.1621114	12	0.34684
Within Subjects	159.0220933	28	5.67936
B	77.3918586	2	38.6592
AB Interaction	36.2448605	2	18.12243
BX Subj. W. Group	45.3853743	24	1.8196
FA	116.232545*/4.75 S		
FB	20.462590*/3.40 S		
FAB	9.583227*/3.40 S		

*P < 0.05

Figure 2. ^{131}I-thyroxine degradation regression line and associated parameters of female mink controls not receiving polychlorinated biphenyl.

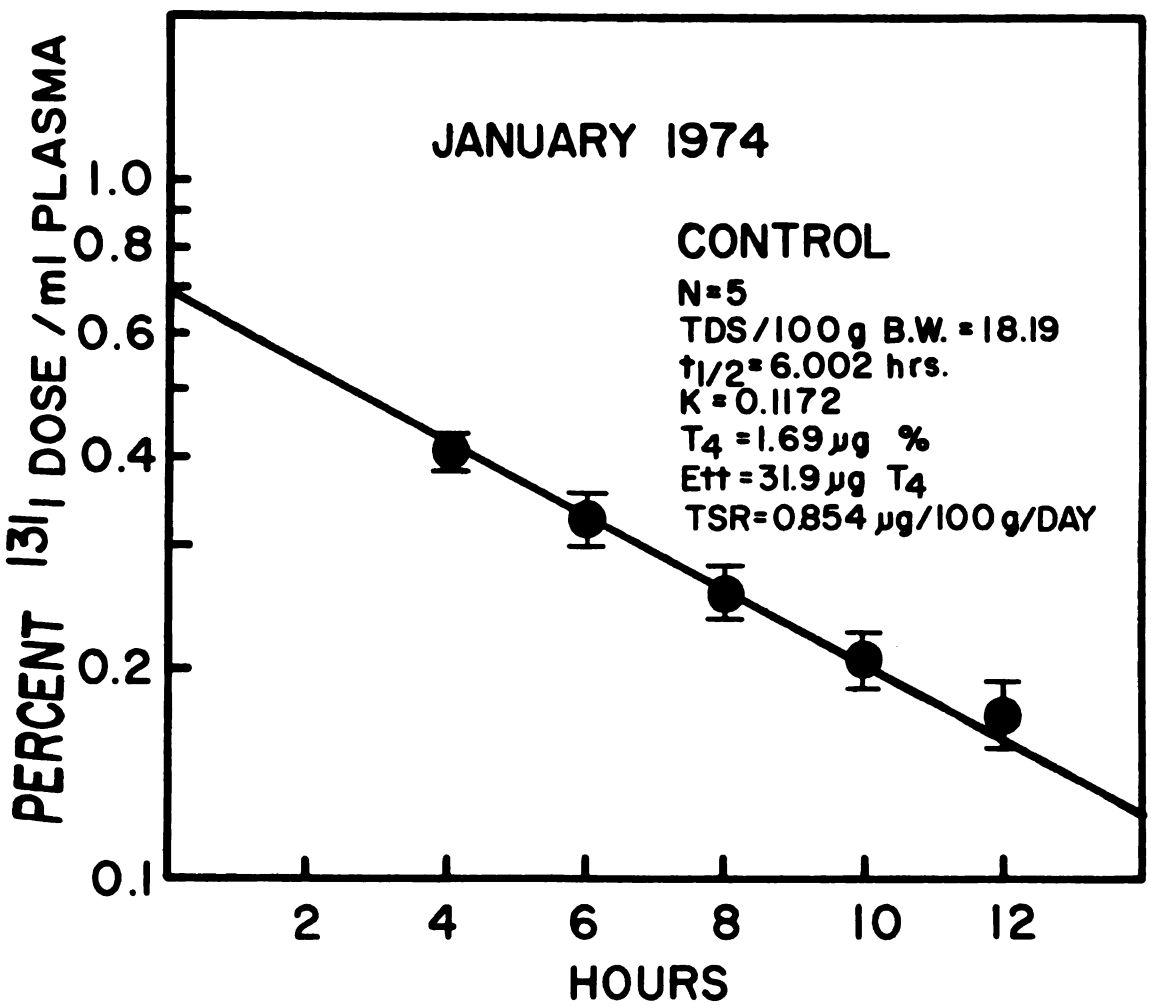

JANUARY 1974
CONTROL
N=5
TDS/100g B.W. = 18.19
t1/2=6.002 hrs.
K=0.1172
T4 = 1.69 µg %
Ett = 31.9 µg T4
TSR=0.854 µg/100g/DAY
PERCENT 131I DOSE / ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 2

equaled 6.002 hours. Thyroxine distribution space (TDS/ 100 gm body weight) equaled 18.19 mls. The total amount of thyroxine present in all of the pools outside of the thyroid, extrathroidal thyroxine (Ett) equaled 31.9 µg.

By February 22nd (see Figure 3), thyroxine secretion rate of the controls was 0.44 µg/100 g/day which is approximately one-half that observed in the other group of controls in experiment II sampled in January. The 5 ppm PCB group was found to be over 5 times higher than the control at 2.35 µg/100 g/day. Plasma thyroxine at 3.69 µg/100 ml in the 5 ppm PCB group was over 2.5 times higher than controls at 1.28 µg/100 ml. Degradation rate constant, K was significantly greater in the 5 ppm PCB group than the controls (0.1338 and 0.1109, respectively), and although the slopes do not appear visually different, they are significantly different (see Summary Table 5). Biological half-life also was significantly shorter in the 5 ppm group than controls 5.16 hrs. vs. 6.17 hrs. respectively. TDS/100 g body weight was also significantly larger in the 5 ppm PCB group than controls, 20.04 ml vs. 12.68 ml respectively. Ett was approximately four times larger in the 5 ppm PCB group than it was in the controls, 66.7 µg T_4 vs. 16.46 µg T_4 respectively.

During mid-gestation in April (4-12-74), TSR in both groups decreased significantly from their previous sampling date in February (see Figure 4). The 5 ppm PCB group TSR

Figure 3. ^{131}I-thyroxine degradation regression line and associated parameters of pre-estrus female mink controls and those receiving 5 ppm polychlorinated biphenyl.

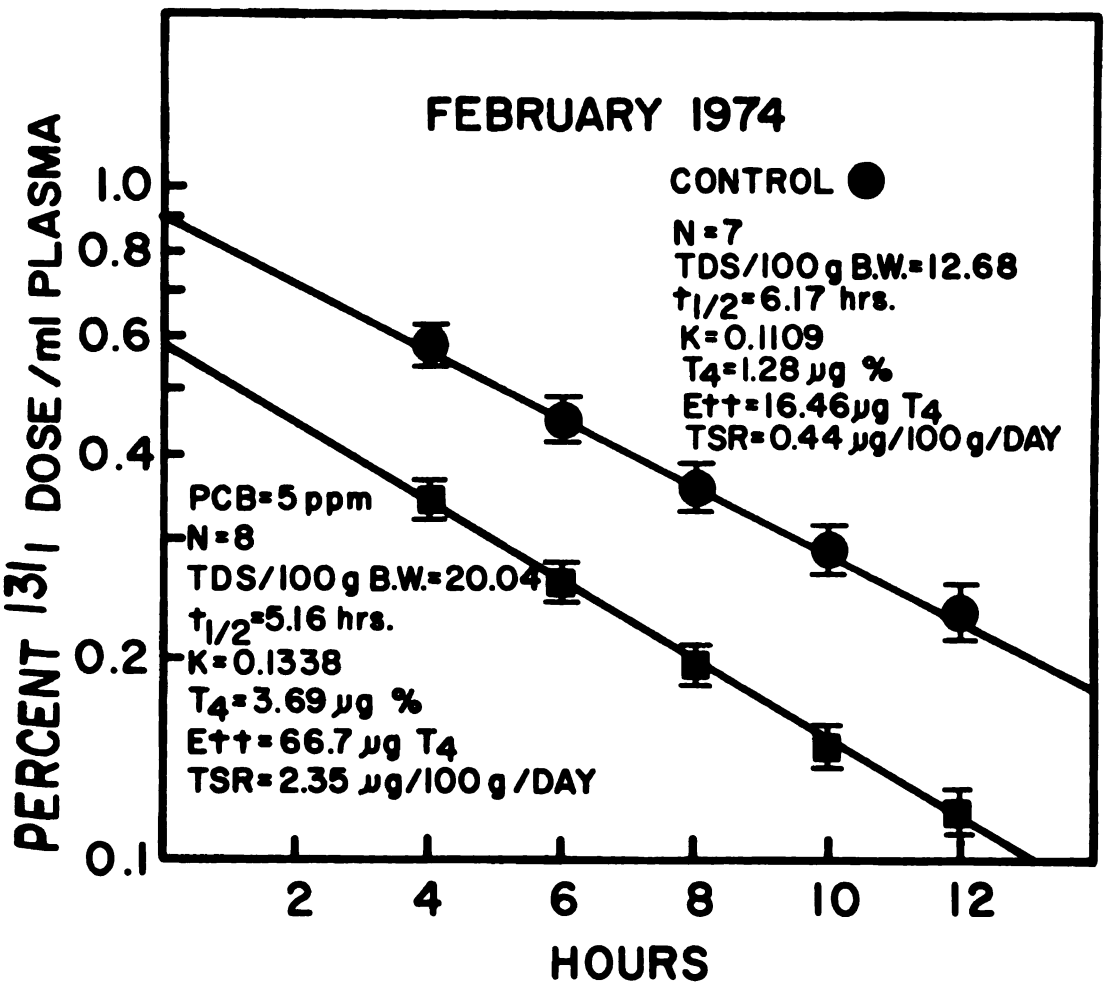

FEBRUARY 1974
CONTROL
N=7
TDS/100g B.W.=12.68
t1/2=6.17 hrs.
K=0.1109
T4=1.28 µg %
Ett=16.46µg T4
TSR=0.44 µg/100g/DAY
PCB=5 ppm
N=8
TDS/100g B.W.=20.04
t1/2=5.16 hrs.
K=0.1338
T4=3.69 µg %
Ett=66.7 µg T4
TSR=2.35 µg/100g/DAY
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 3

Table 5. Statistical Comparison of Data in Experiment II (TSR-TDR) Using the Scheffé F Ratio

Treatment Comparison (A Effect)					
MS		Feb.	Apr.	May (L)	May (NL)
15.622	TDS F=	121.26	21.93	1.22 NS	35.59
0.7634	$T_{\frac{1}{2}}$	46.72	242.1	88.49	231.0
0.0037	K	25.4	145.9	10.40	35.0
0.1816	T_4	11.3	220.0	220.0	132.64
0.0206	ETT	4.28	49.64	123.76	184.8
0.3468	TSR	36.78	68.23	75.50	8.58

MS Subj. W. Group; $V_1 = 1$, $V_2 = 12$

$P < 0.05$ Critical Value = 4.75

continued

Table 5--continued

Time Comparison (B Effect)

	TDS (MS - 15.804)			$T_{\frac{1}{2}}$ (MS = 1.2926)		
PCB	Feb. vs. April	28.30	PCB	Feb. vs. April	0.520	NS
PCB	May vs. April	108.02	PCB	May vs. April	14.57	
PCB	May vs. Feb.	25.74	PCB	Feb. vs. May	9.58	
C	Jan. vs. Feb.	13.49	C	Feb. vs. April	5.20	
C	Feb. vs. April	38.11	C	April vs. May NL	13.69	
C	Feb. vs. May L	46.24	C	April vs. May L	5.58	
C	Feb. vs. May NL	53.38	C	Feb. vs. May L	35.77	
C	April vs. May L	96.19				
C	May vs. Jan.	13.20				

	K (MS = 0.0007)			T_4 (MS = 0.1883		
PAC	Feb. vs. April	14.14	PCB	Feb. vs. April	36.33	
PCB	May vs. April	14.65	PCB	May vs. Feb.	18.23	
PCB	May vs. Feb.	13.59	PCB	May vs. Feb.	13.60	
C	Feb. vs. April	195.	C	Jan. vs. Feb.	7.03	
C	April vs. May L	1.07	NS C	Feb. vs. April	0.506	NS
C	April vs. May NL	3.47	NS C	Feb. vs. May L	24.81	
C	Feb. vs. May L	244.4	C	Feb. vs. May NL	42.20	
C	Jan. vs. Feb.	0.390	NS C	April vs. May L	18.23	
				April vs. May NL	33.90	
				Jan. vs. May L	5.42	
				Jan. vs. May NL	15.07	

MS BX Sub. W. Group; V = 2, V = 24

P < 0.05 Critical Value = 4.32

continued

Table 5--continued

	ETT (MS = 0.0215)			TSR (MS = 0.1819)		
PCB	Feb. vs. April	44.71	PCB	Feb. vs. April	76.05	
PCB	May vs. April	105.83	PCB	May vs. April	38.57	
PCB	May vs. Feb.	13.029	PCB	May vs. Feb.	6.53	
C	Jan. vs. Feb.	7.94	C	Jan. vs. Feb.	6.34	
C	Feb. vs. April	0.499	C	Feb. vs. April	0.82	NS
C	Feb. vs. May NL	47.42	C	Feb. vs. May L	24.29	
C	April vs. May NL	55.38	C	Feb. vs. May NL	11.90	
C	May vs. Jan. NL	16.19	C	April vs. May NL	18.98	
				Jan. vs. May NL	0.86	NS
				Jan. vs. May L	5.808	

MS BX Subj. W. Group; $V_1 = 2$, $V_2 = 24$

$P < 0.05$ Critical Value = 4.32

Figure 4. ^{131}I-thyroxine degradation regression line and associated parameters of pregnant female mink controls and those receiving 5 ppm polychlorinated biphenyl.

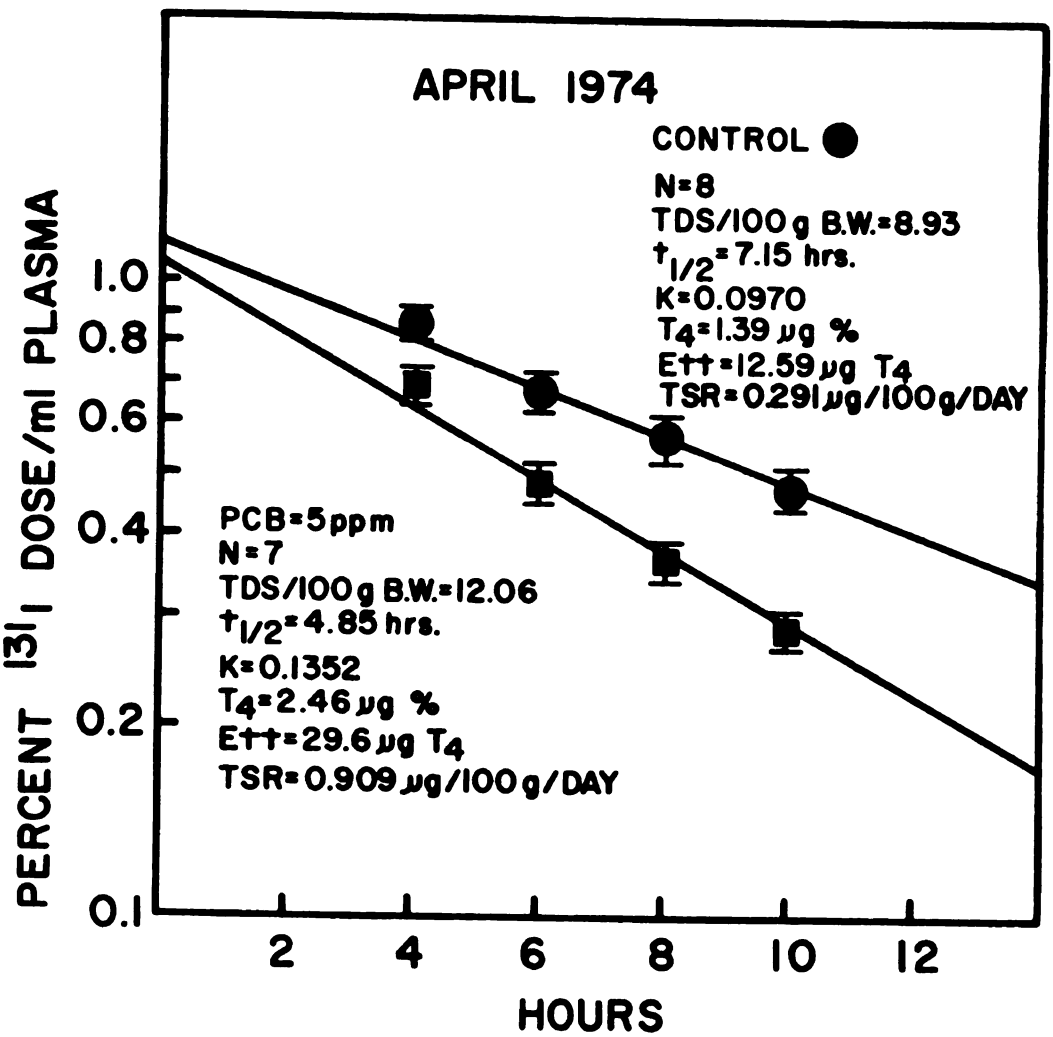

APRIL 1974
CONTROL
N=8
TDS/100g B.W.=8.93
t1/2=7.15 hrs.
K=0.0970
T4=1.39 µg %
Ett=12.59 µg T4
TSR=0.291 µg/100g/DAY
PCB=5ppm
N=7
TDS/100g B.W.=12.06
t1/2=4.85 hrs.
K=0.1352
T4=2.46 µg %
Ett=29.6 µg T4
TSR=0.909 µg/100g/DAY
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 4

group decreased by approximately 2.5 times to 0.909 µg/100 g/day and the controls decreased 30% to 0.291 µg/100 g/day but a three-fold difference was still observed between the control and 5 ppm PCB group. The small increase in T_4 between controls in February and April was not significant but the difference between the 5 ppm PCB group at 2.46 µg percent and control of 1.39 µg percent was significant. The rate constant K decreased significantly between February and April to 0.0970 in the controls but increased slightly but significantly to 0.1352 in the 5 ppm PCB group. The 5 ppm PCB group was significantly higher than the controls in April. The $t_{\frac{1}{2}}$'s lengthened significantly to 7.15 hrs. in the controls between February and April and decreased in the 5 ppm PCB group to 4.85 hrs. The 5 ppm PCB group's $t_{\frac{1}{2}}$ was significantly shorter than in the controls.

The TDS/100 g body weight decreased significantly in both controls and 5 ppm PCB group to 8.93 ml and 12.6 ml respectively between February and April. The 5 ppm PCB group was significantly higher than the controls. Ett in the 5 ppm PCB group was reduced to 29.6 µg T_4 which is one-half of its February level. Control Ett also decreased significantly to 12.59 µg T_4. Ett of the 5 ppm group remained significantly higher than in the control.

At mid-lactation in May (5-16-74) (see Figure 5), TSR in the control mink, both lactating and non-lactating, equaled 1.25 and 1.00% µg/100 g/day, respectively.

Figure 5. ^{131}I-thyroxine degradation regression line and associated parameters of lactating and non-lactating female mink controls and those receiving 5 ppm polychlorinated biphenyl.

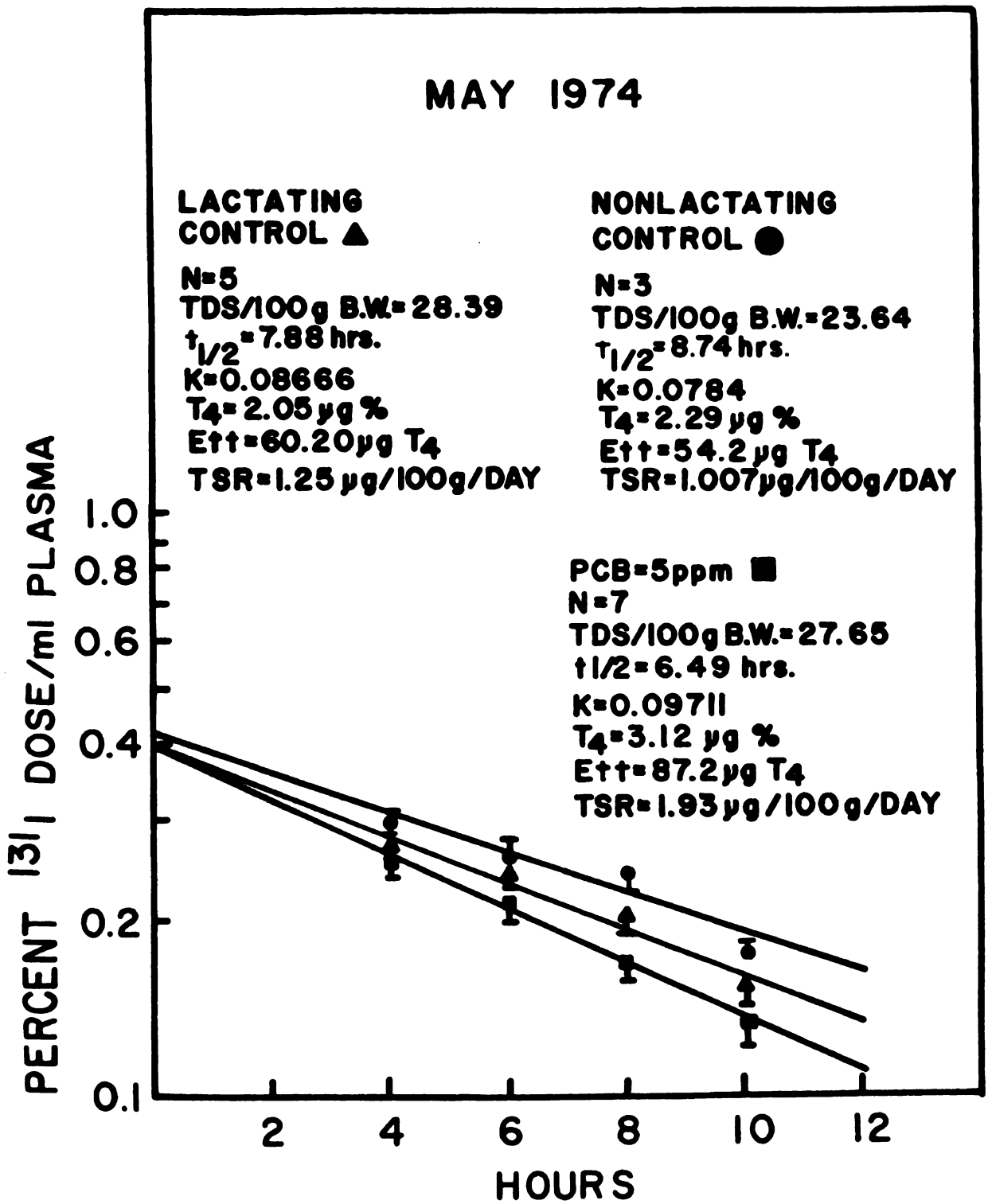

MAY 1974
LACTATING
CONTROL ▲
N=5
TDS/100g B.W.=28.39
t1/2=7.88 hrs.
K=0.08666
T4=2.05 µg %
Ett=60.20 µg T4
TSR=1.25 µg/100g/DAY
NONLACTATING
CONTROL ●
N=3
TDS/100g B.W.=23.64
T1/2=8.74 hrs.
K=0.0784
T4=2.29 µg %
Ett=54.2 µg T4
TSR=1.007 µg/100g/DAY
PCB=5ppm ■
N=7
TDS/100g B.W.=27.65
t1/2=6.49 hrs.
K=0.09711
T4=3.12 µg %
Ett=87.2 µg T4
TSR=1.93 µg/100g/DAY
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 5

The control TSR values were significantly higher than at any other time in the study. The TSR's during lactation increased over the gestational rate in April by a factor of 4 in lactating controls and a factor of 3 for the non-lactating controls. The TSR of the 5 ppm PCB group doubled from April to 1.93 µg/100 g/day which is still significantly higher than both lactating and non-lactating control groups in Experiment II. In all the parameters measured between the two control groups in May in Experiment II, only non-significant differences were found. The plasma T_4 level increased significantly between April and May to 2.05 µg percent for lactating and 2.29 µg percent for non-lactating controls. T_4 levels of the PCB groups significantly increased to 3.12% which is still significantly lower than the high observed in January of 3.69 µg percent. T_4 levels of both control groups were found to be significantly lower than in the treated group. T½ increased significantly to a rate higher than at any other time in both lactating (7.88 hrs.) and non-lactating (8.74 hrs.) controls; however, the difference between the two controls were not significant. The 5 ppm PCB groups t½ increased significantly between April and May but remained significantly lower than in February when the most rapid rate of degradation and secretion was observed. The 5 ppm PCB groups t½ (6.49 hrs.) remained significantly lower than the controls.

No significant changes occurred in rate constants K in the controls between April and May but at 0.0866 for lactating controls and 0.0784 for non-lactating controls they were significantly lower than the K = 0.1109 for controls in February. The rate constant of the 5 ppm PCB group at 0.0971 was significantly lower than in April but was significantly higher than in both controls in May.

TDS in May controls increased significantly over the other sampling dates to 28.39 ml/100 g body weight for lactating and 23,64 for non-lactating groups. The 5 ppm PCB group's TDS doubled between April and May to 27.65 ml which is significantly higher than in the non-lactating controls but not significantly different from the lactating control.

Ett in controls, during May (60.24 µg T_4 lactating, 54.2 µg T_4 non-lactating) increased to 4 times the April level of 12.59 µg T_4. The 5 ppm PCB group's Ett increased in May to 87.2 µg T_4 which is 3 times its April level and significantly higher than in the two control groups (see Appendix E for data summarized in Figures 2-7 and Table I).

A summary of the degradation slopes may be observed for the controls in Figure 6 and for the 5 ppm PCB group in Figure 7.

Figure 6. Summary of ^{131}I-thyroxine degradation regression lines for control mink.

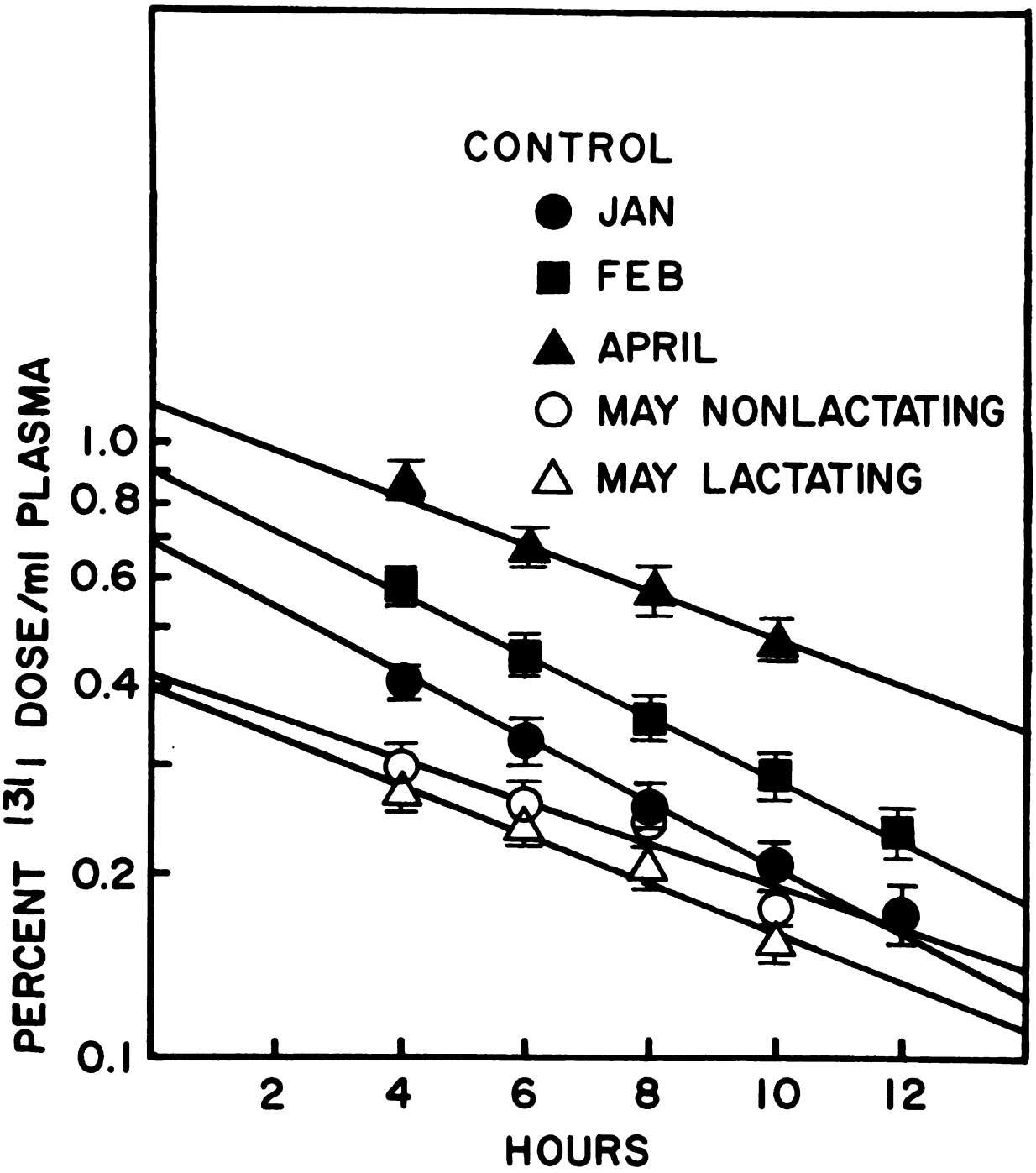

CONTROL
JAN
FEB
APRIL
MAY NONLACTATING
MAY LACTATING
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 6

Figure 7. Summary of ^{131}I-thyroxine degradation regression lines for mink receiving 5 ppm polychlorinated biphenyl.

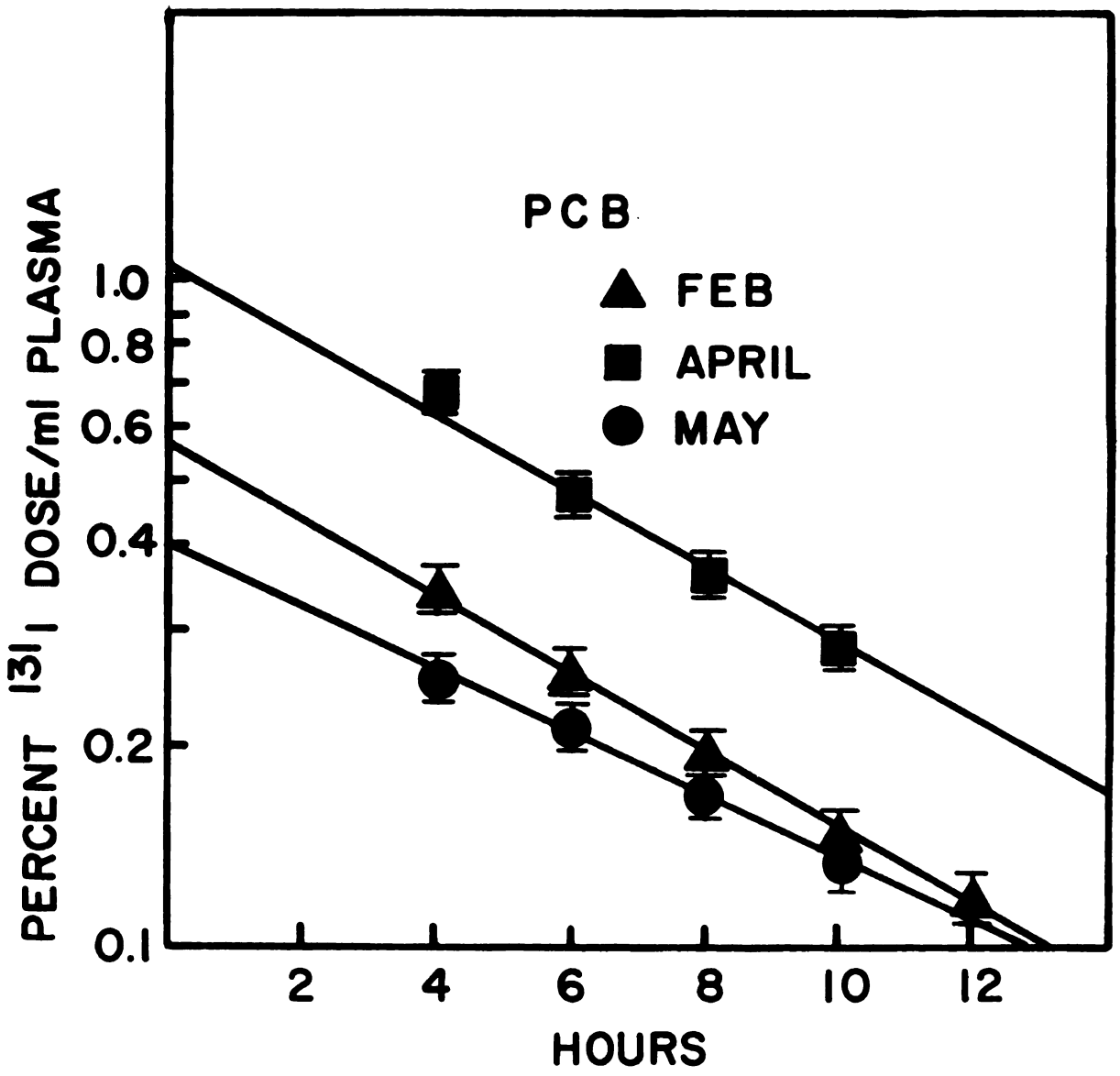

PCB
FEB
APRIL
MAY
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 7

Figure 7. Summary of ^{131}I-thyroxine degradation regression lines for mink receiving 5 ppm polychlorinated biphenyl.

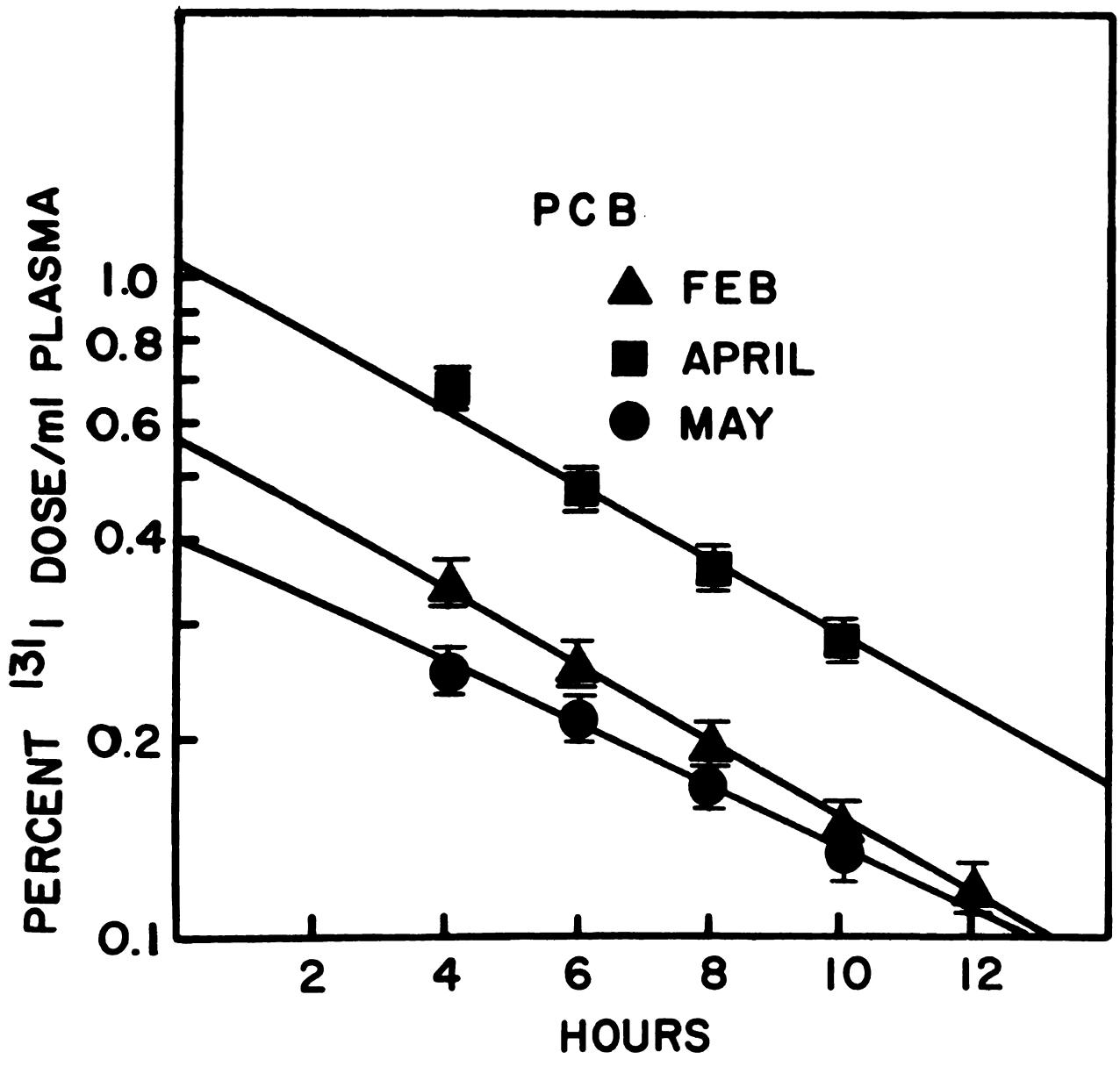

PCB
FEB
APRIL
MAY
PERCENT 131I DOSE/ml PLASMA
1.0
0.8
0.6
0.4
0.2
0.1
2
4
6
8
10
12
HOURS

Figure 7

Experiment III--Male and Female Thyroxine Levels

Male and female mink plasma thyroxine was found to be significantly different only in July, 1973 (see Figure 8), when the female T_4 level was 2.52 µg percent and the male was 1.72 µg percent. Male T_4 levels were highest in February and females were highest in July at 2.52 µg percent. Male T_4's were higher, though not significantly so, than in females in the months of August, February and April whereas females' T_4 was higher but not significantly, than males in December, March, and May (see Appendix F for data summarized in Figure 8). Seasonal changes were observed in both males and females.

Experiment IV--Thyroxine Binding and Saturation Index

The thyroxine binding curve in Figure 9 shows that a plateau occurs at approximately 2.00 µg percent T_4 which indicates that December mink saturate their TBG at 2 µg percent; however, more importantly, the curve reveals the quantity of exogenous and endogenous thyroxine necessary to saturate the thyroxine binding globulin without exceeding the conditions of pH 8.6 barbital buffer and suppression of binding to prealbumins and albumin.

The binding plateau remains constant to approximately 26 µg percent whereupon it rises steeply. Binding to other proteins probably occurs at T_4 concentrations above 26 µg percent T_4, most likely to thyroxine-binding pre-albumins

Figure 8. Comparison of plasma thyroxine (µg/100 ml plasma) of male and female mink measured at periodic intervals of the year.

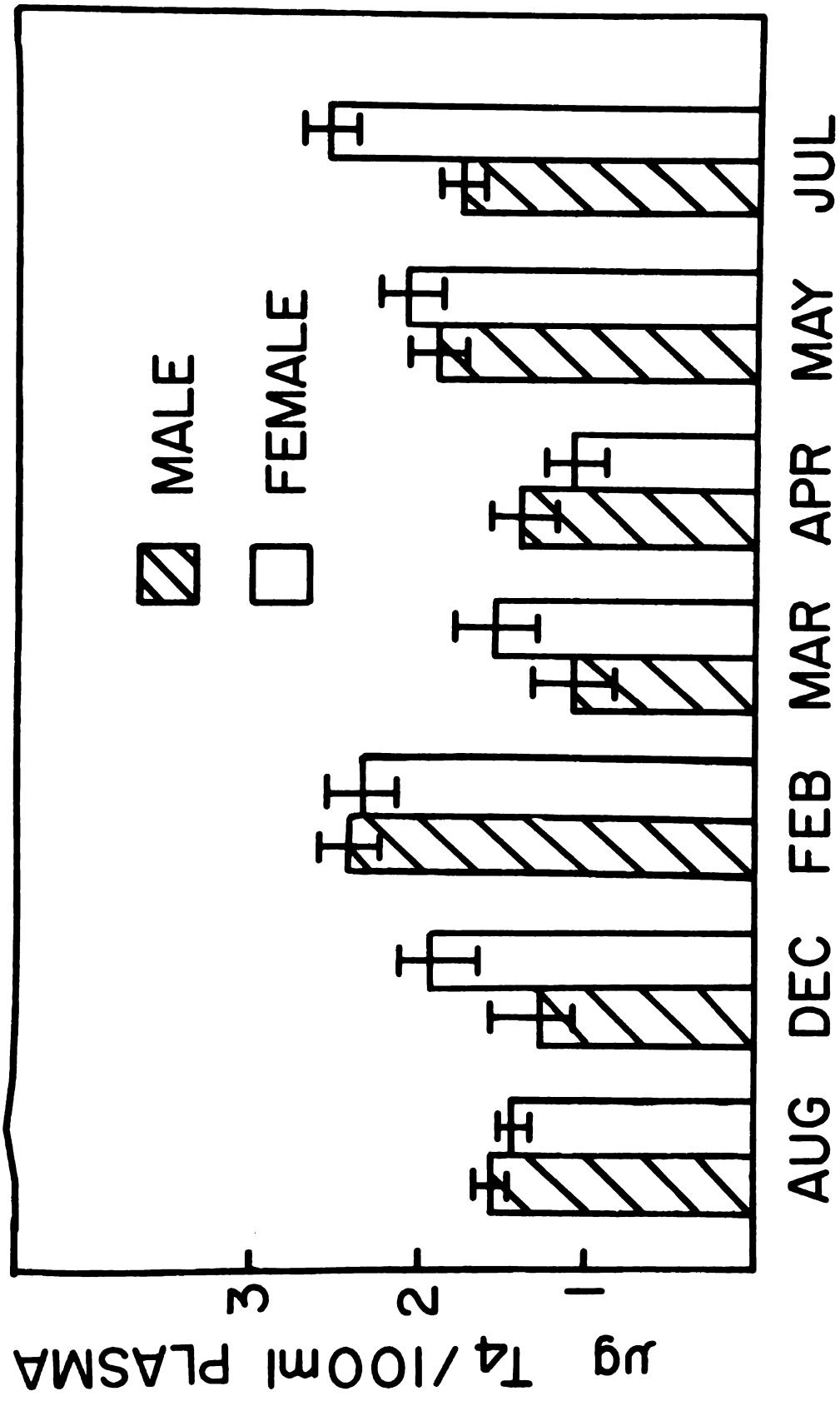


Figure 8

Figure 9. Thyroxine binding curve of plasma measured on five mink in December. Each point represents the mean ± standard error for thyroxine (µg/100 ml plasma) bound to protein.

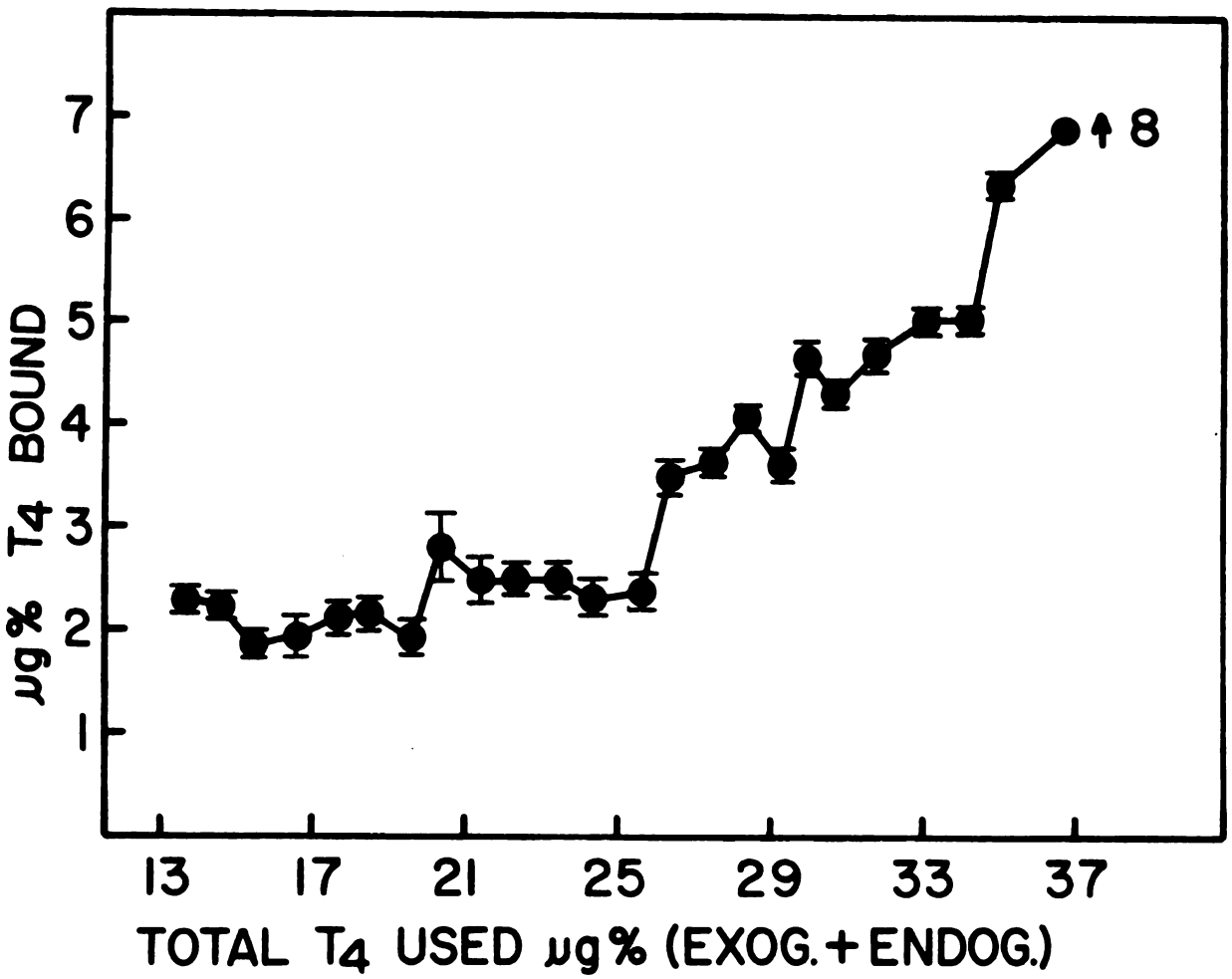

7
6
5
4
3
2
1
µg% T4 BOUND
8
13
17
21
25
29
33
37
TOTAL T4 USED µg% (EXOG.+ENDOG.)

Figure 9

(TBPA) and albumins. Therefore, to remain within the limits of the thyroxine binding globulin capacity, 19 µg percent total T_4 (exogenous and endogenous) was used to assess the saturation of the control and 5 ppm PCB treatment animals.

The saturation index (SI) (T_4 binding capacity of TBG divided into the plasma T_4 level) of the 5 ppm PCB group was significantly higher than the control at each sampling date (see Figure 10 and Table 6). During pre-estrus, in February, the saturation index of the 5 ppm PCB group at 3.0 was three-fold higher than the controls at 1.0. Between February and gestation, in April, there was a significant decrease in both the controls and the 5 ppm PCB group SI. The 5 ppm PCB group saturation index dropped by one-half to 1.318 and the control dropped significantly to 0.531 in April. Despite the decrease in SI, the 5 ppm PCB group remained significantly higher than the controls.

Between gestation in April and lactation in May no significant changes occurred in SI although both the 5 ppm PCB and control groups increased slightly to 1.441 and 0.634 respectively. The 5 ppm PCB group remained significantly higher than the controls. TBG-capacity increased significantly in April and remained high during lactation in May.

Reproductive Performance

The reproductive rate for mink on 5 ppm and 2 ppm PCB diets in experiments I and II is essentially zero. One kit

Figure 10. Saturation index of plasma from female mink. SI = plasma thyroxine level in µg/100 ml plasma/ thyroxine binding globulin capacity.

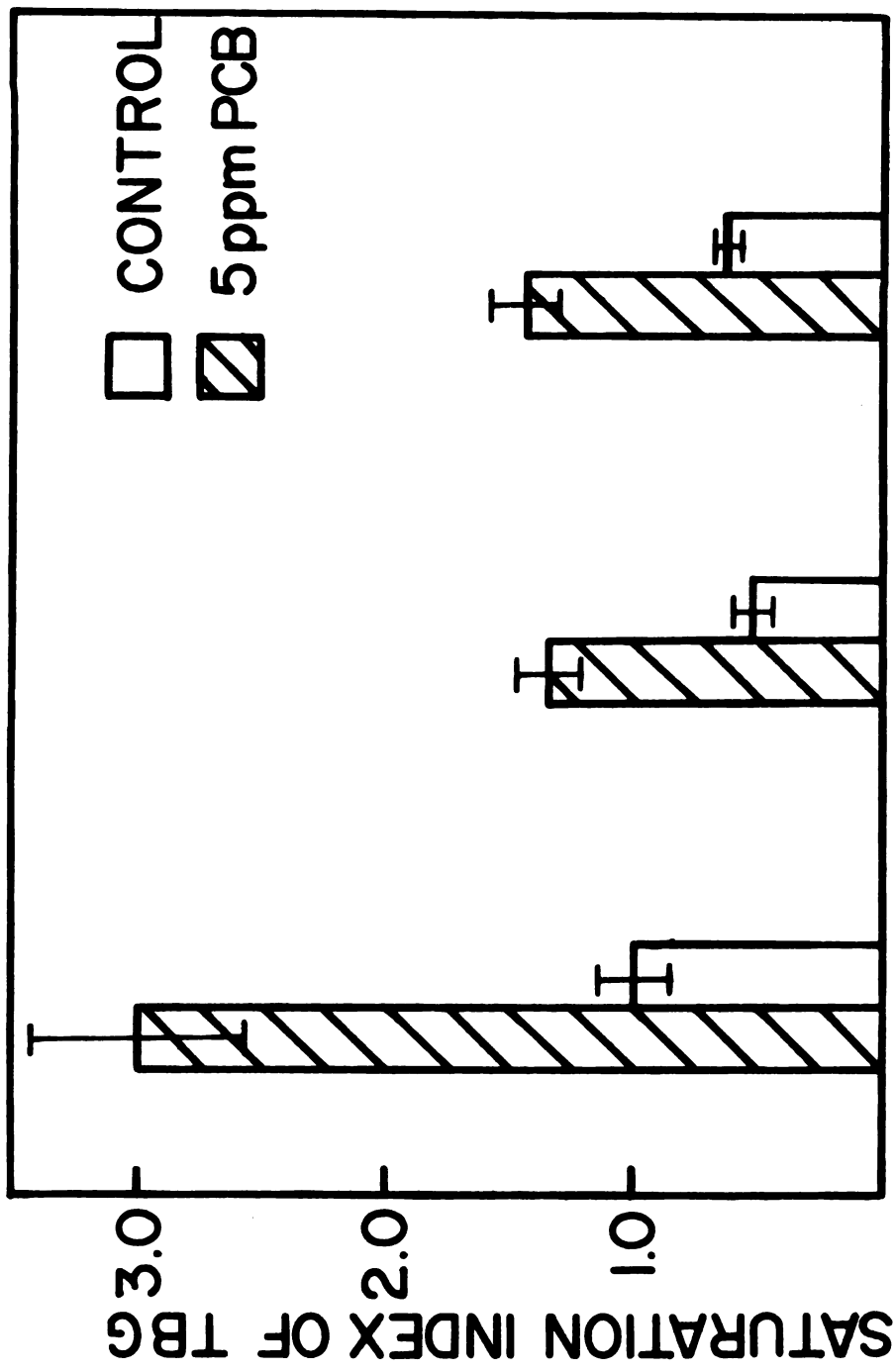


Figure 10

Table 6. Saturation Index (SI) and TBG-capacity of Thyroxine Binding Globulin (Experiment IV)

Animal Number	February			April			May		
	T_4 µg percent	T_4 binding capacity	SI	T_4 µg percent	T_4 binding capacity	SI	T_4 µg percent	T_4 Binding capacity	SI
5 ppm PCB									
A120	3.67	2.06	1.78	2.25	2.31	0.97	3.26	1.89	1.72
A620	4.34	1.23	3.52	2.98	1.76	1.69	3.05	2.97	1.02
A602	3.59	3.73	0.96	-	-	-	3.81	3.26	1.18
A690	3.13	0.57	5.49	1.89	1.29	1.46	3.30	1.89	1.74
A64	4.23	2.14	1.97	1.80	2.19	0.82	3.58	1.82	1.96
A1244	3.92	1.59	2.46	2.21	1.92	1.15	2.85	3.28	0.86
A580	3.32	1.04	3.19	2.68	2.79	0.96	1.99	1.25	1.59
A700	3.37	0.72	4.68	3.24	1.73	2.16	-	-	-
$\bar{X}$	3.57	1.63	3.00	2.50	1.99	1.31	3.12	2.33	1.44
S.E.	0.12	0.36	0.54	0.25	0.18	0.18	0.22	0.30	0.15
Controls									
Q2376	1.04	0.90	1.18	1.19	2.68	0.36	1.63	1.76	0.58
T160	1.73	0.96	1.80	1.65	3.14	0.52	2.12	3.55	0.59
T440	1.84	2.39	0.77	1.29	2.71	0.59	2.17	5.75	0.37
T416	0.98	1.12	0.88	1.11	4.09	0.27	2.13	2.97	0.71
T240	1.43	1.70	0.84	1.05	1.67	0.62	2.26	3.50	0.64
R620	0.69	1.22	0.56	1.82	1.86	0.97	2.59	2.98	0.86
R1030	1.22	1.17	1.04	1.83	5.16	0.35	2.12	3.59	0.59
Q2044	-	-	-	-	-	-	2.10	2.99	0.70
$\bar{X}$	1.27	1.16	1.00	1.42	3.03	0.53	2.14	3.51	0.63
S.E.	0.15	0.30	0.15	0.12	0.46	0.08	0.09	0.33	0.04

was produced by 23 animals and it died. All of the animals in experiments I and II were mated and motile sperm were found in the vagina. The 0.5 ppm PCB group significantly outproduced the controls in both experiments I and II. There were twice as many kits produced by the 0.5 ppm PCB group as in the controls (16 and 35 respectively at birth). For various reasons, after 8 weeks only three control kits were alive, while the 0.5 ppm PCB group had weaned 15 kits.

Eight control mink in experiment II produced 26 kits but since the adults were killed for autopsy, the young were farmed out to adoptive mothers. (See Table 7.)

Anatomical Parameters

It was found that mink from experiment II, when autopsied on May 18, 1974 (see Table 8), had thyroid weights not significantly different between controls and the 5 ppm PCB group. Liver weights were significantly higher in the PCB group, 28.78 gm vs. 27.45 gm for lactating controls and 24.04 gm for non-lactating controls. When expressed as grams liver weight/100 gm body weight, the differences are still significant (3.68 gm for the PCB group and 2.94 and 2.53 gm for lactating and non-lactating controls). Differences in body weight were not significant. The PCB group had adrenal weights (11.32 gm/100 gm body weight) significantly higher than the controls (8.19 gm for lactating and 6.88 gm for non-lactating). Body weights did not

Table 7. Reproduction of Mink in Experiments I and II

Experiment I	Number females mated	Number females to give birth	Kits observed alive at birth	Kits alive at 8 weeks
Control	7	5	16	3
0.5 ppm	8	7	35	15
2 ppm	7	4	1	0
5 ppm	8	0	0	0
Experiment II				
Control	7	6	26	*
5 ppm	8	0	0	*

(N = 8 for each group except controls in experiment I where there were 7 females.)

*Adults were autopsied and kits were placed with foster mothers.

Table 8. Body Weights and Organ Weights of Mink from Experiment II (Autopsied May 18, 1974)

Mink Number	Body Wt. (g)	Liver Wt. (g)	Liver Wt. (g) 100 g B.W.	Thyroid Wt. (Lt.+Rt.) mg	Thyroid Wt. 100 g B.W.	Adrenal Wt. (Lt.+Rt.) (g)	Adrenal Wt. 100 g B.W.
5 ppm PCB							
A120	884	23.35	2.64	52	5.89	101	11.42
A580	677	30.79	4.55	32	4.73	84	12.44
A620	843	31.06	3.72	53	6.29	102	12.09
A602	820	31.20	3.80	47	5.16	74	9.60
A690	1001	32.88	3.28	57	5.66	122	12.24
A700	819	39.27	4.79	48	5.86	100	12.20
A1244	699	21.77	3.11	45	6.44	90	12.90
A64	793	27.93	3.52	40	5.04	61	7.69
X	817	28.78	3.68	46.7	5.71	92.3	11.32
S.E.	36	1.79	0.25	2.7	0.20	6.4	0.62
Lactating Control							
Q2376	1071	27.51	2.57	27	2.60	81	7.55
T240	980	29.55	3.02	48	4.97	82	8.37
T160	834	27.30	3.27	44	5.28	102	12.23
A416	873	25.45	2.91	43	4.93	61	7.01
X	939	27.45	2.94	40.7	4.44	81.4	8.79
S.E.	53	0.84	0.14	4.4	0.62	8.3	1.18
Nonlactating Control							
R620	1039	22.48	2.16	54	5.27	66.6	6.40
T440	1148	26.48	2.31	69	5.99	59.8	5.21
R1030	748	23.32	3.21	49	6.58	61.5	9.02
X	978	24.04	2.53	57.3	5.95	64.6	6.88
S.E.	119	1.22	0.29	6.0	0.38	2.4	1.12

significantly differ in May, between controls and 5 ppm PCB group animals. Body weights did differ with time in both experiment I and experiment II (see Appendices E and G for body weight by date). Analysis of variance of body weights of animals in experiment II may be seen in Table 4, where no significant differences exist between treatments but do exist for time and time-treatment interaction.

Cursory examination of the heart, stomach, intestines, lungs, liver, brain and pituitary of the 16 mink autopsied from experiment II, revealed no obvious differences between the controls and the PCB group.

Despite the lack of significant differences in thyroid weights, histological examination shows that thyroids of PCB group animals were more active than the controls. The PCB group had taller follicular cells generally larger follicles and more vacuoles in the colloid than controls (see Figures 11 and 12 for photo micrographs of the PCB group and control thyroids).

Figure 11. Thyroid gland photomicrograph from a control mink during mid-lactation (4-18-74) (magnified 250 times).

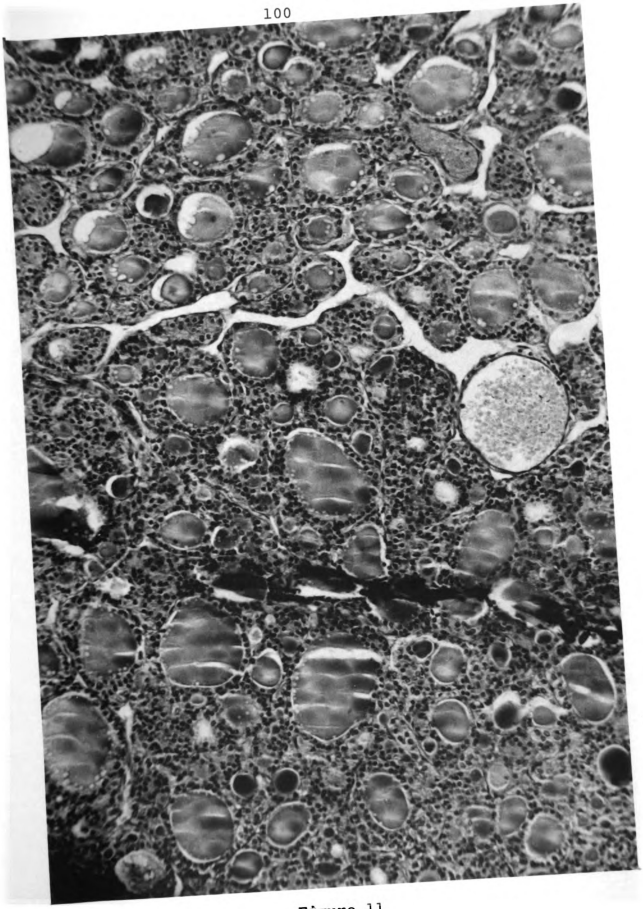


Figure 11

Figure 12. Thyroid gland photomicrograph from a female mink receiving 5 ppm polychlorinated biphenyl (4-18-74) (magnified 250 times).

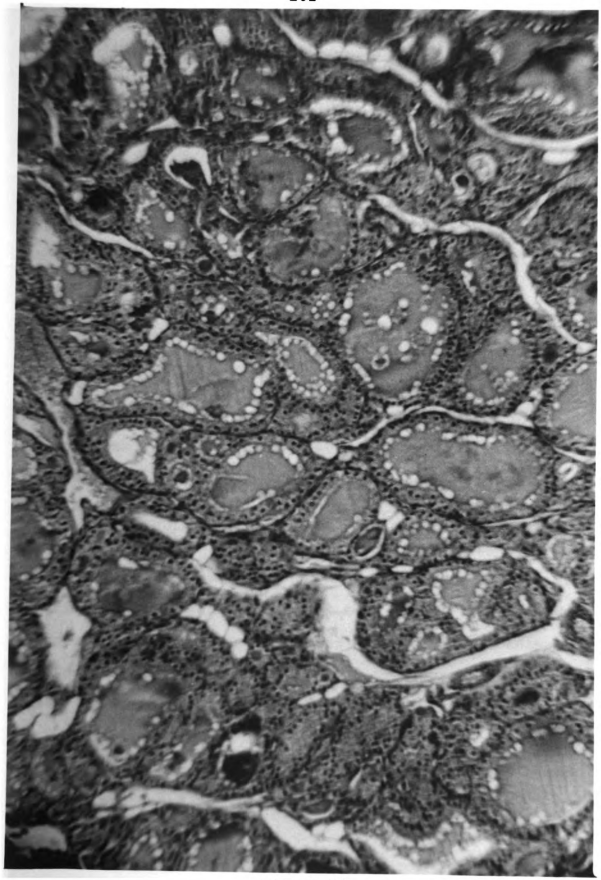


Figure 12

Detail on f. 112

DISCUSSION

Generally PCB's have an overall stimulating effect upon thyroid function in mink except during reproduction, when dosages and events modify thyroid and thyroxine hormone parameters. T_4 modifications by PCB's at reproduction apparently affect the biological response to breeding and its associated biochemical events.

Feeding PCB's for a long term at 5 ppm and 2 ppm results in stimulation of thyroxine secretion rate (TSR) which occurs throughout most of the winter and spring months; however, TSR becomes quite low by mid-pregnancy. During the time of high estrogen in April, the degradation rate (rate constant K) remains elevated in the 5 ppm PCB group (Figure 4). This creates a condition of high utilization with concomitantly lower secretion. The result is seen in Experiment I (Figure 1) as a drop in T_4 level in the 5 ppm group at the time of high estrogen level. Control animals, particularly in Experiment I, responded oppositely. Circulatory T_4 was elevated during estrus, implantation and also early gestation (Figure 1). Through most of the autumn and winter in Experiment I, very high T_4 levels were found in the 5 ppm and 2 ppm PCB groups which may indicate a high

thyroxine secretion rate as seen during mid-February in Experiment II (Figure 3). TSR was probably high in the PCB-treated animals throughout the diestrus months, since the T_4 level was high (Figure 1).

Interestingly, the very high T_4 levels in the face of high secretion rate indicate a probable malfunction of the normal feedback system involved in TSH release. T_4 levels are sufficiently high to completely saturate the TBG and also to precipitate binding of T_4 to other carriers such as prealbumin and albumin (Table 6). The saturation index of the carrier proteins with T_4 in the 5 ppm PCB groups is three times that of the controls in February (Table 6) and exceeds the thyroxine binding globulin capacity by a factor of three. Since the TBG capacity in the 5 ppm PCB group is essentially equal to the control TBG capacity high T_4 levels have not stimulated increases in the amount of circulating TBG. Carrying capacity is probably expanded by the presence of other carriers. The feedback mechanism, if it were operating normally, would still be expected to respond to the high free T_4 levels which exist under such excess T_4 conditions.

Estrus and pregnancy and their associated behavior and hormonal changes alter thyroid functions in both PCB groups and controls. At implantation and early gestation when estrogen levels are very high in most animals, T_4 was high

in the controls (Figure 1) but was low in the 5 ppm groups (Figure 1 and Figure 4) two weeks later in mid-pregnancy. Decreases in T_4 and TSR are contrary to observations in other species such as cattle and guinea pigs (Hernandez et al., 1972; Pals, Shaw, and Reineke, 1973) where T_4 and TSH are observed to increase during pregnancy.

A drop in the saturation index of both control and PCB groups to one-half their pre-estrus level was also observed during pregnancy (Figure 10 and Table 6). Part of the decrease may be attributed to (1) a more than two-fold decrease in TSR, which in turn was probably reduced due to a reduction in TSH, and (2) to a large increase in TBG-capacity from 1.16 to 3.0 µg percent may have been stimulated by estrogen and progesterone.

Serum thyroxine decreased throughout pregnancy in the controls which is opposite to that observed in such species as rats and humans (Heineman et al., 1948; Man et al., 1969; Robbins & Nelson, 1958). The T_4 in the PCB group (Figure 1) was not similarly affected since it had not risen during estrus. The very low T_4 levels probably contributed in part to the high mortality of the fetuses. Ringer et al. (1972) reported low reproductivity in mink on 5 ppm PCB; furthermore, they observed that mink receiving PCB long term at 10 ppm had high maternal mortality.

Estrogen is known to increase thyroid activity. Soliman and Reineke (1955) have shown that estrogen or progesterone plus estrogen when administered to ovarectomized rats stimulate ^{131}I uptake by the thyroid. Progesterone alone had quite the opposite effect. It reduced ^{131}I uptake by the thyroid. For estrogen to affect thyroid function they have found that the pituitary and its interrelationship with the CNS must remain intact and functional. Progesterone probably functions similarly. No information is available concerning the tidal changes in steroid hormone levels during mink reproduction; however, there must be some circulating estrogen and progesterone in the PCB-fed groups since ovulation and some implantation is known to occur. It is not known whether PCB-fed animals in this stage were implanting but observation of the mink by experienced mink breeders failed to reveal any externally visible signs of advancing pregnancy in PCB treated mink.

Despite some apparently normal behavioral estrogenic effects in PCB mink, no increased thyroid activity was observed at estrus or proestrus as occurred in the controls and many other species such as rats, mice, and humans (Schreiber, 1967; Hotelling and Sherwood, 1971). In fact the decrease in thyroxine levels which occurred during estrus (Figure 1, Figure 4) in the two highest PCB groups indicates that the impaired feedback system is at least in part operable.

Additional supportive evidence of an impaired T_4 negative feedback mechanism in PCB-fed animals was observed with the thyroxine binding globulin. The saturation index in May remained 1.5 times higher than the saturability of TBG (Figure 10, Table 6). This occurred at a time when control TBG was only 63% saturated. This implies a higher than normal free T_4 level since the free or loosely bound to bound T_4 equilibrium is apparently raised.

A noteworthy event occurred with regard to T_4 levels in the 0.5 ppm PCB group 4 (Figure 1). Throughout most of the seasons preceding estrus this group had T_4 levels which could be described as stimulated but not as severely overstimulated as observed in the highest PCB group. At estrus, in March, there was sufficient responsiveness remaining to be highly stimulated by estrus and the reproduction events. The T_4 level of the 0.5 ppm PCB group exceeded that of the control through the reproduction season. T_4 stimulation at this PCB level and at that time of year was apparently reproductively optimal, for they whelped and weaned a significantly large number of young than the controls (Table 7). The excellent birthrate which was observed in mink receiving 0.5 ppm PCB contrasts exceedingly with the zero reproduction observed in the two highest PCB treatment groups. T_4 levels in the 4 groups in Experiment I at implantation (Figure 1) correlate exactly with the reproduction performance observed at parturition (Table 7). Iwamoto (1973) reported that

PCB Aroclor 1254 was slightly stimulatory to reproduction at 1 ppm.

Aulerich et al. (1973), previously reported that high PCB administration inhibits mink reproduction. They have also found that fish from lake Michigan, containing PCB's, reduced reproductive performance. Ringer et al. (1972) and Aulerich et al. (1972) reported that PCB's seem to be more highly concentrated in the brain than in any other organ. Apparently the lipid soluble nature of PCB's causes it to concentrate in fat deposits and in tissue with a high lipid content.

The high content of lipid in the brain is the major reason for the very high PCB concentration found there. It appears likely that the exceedingly high brain PCB levels interfere with CNS operation both humorally and electrically. The high brain PCB appears to further enhance the contention that the alteration in thyroid function may be attributed to the impaired negative feedback mechanism. The feedback system is highly dependent upon the hypothalamic, limbic systems and higher brain centers.

A slightly below normal reproductive rate in controls in Experiment I (Table 7) might be explained by disturbances associated with drawing blood samples and handling during breeding and implantation. These disturbances may have induced some unnoticed abortions.

The fact that thyroxine levels differ between Experiment I and II (Figures 1 and 4) during pregnancy may be explained by the dates on which the samples were taken. Experiment I thyroxine samples were taken on April 6th in early pregnancy; whereas, T_4 samples in Experiment II were taken two weeks later on April 16th. Mink have an approximately 5 week gestation period extending from approximately April 1st to May 7th, and a 10 day difference in sample dates equals approximately one trimester. Since circulating T_4 levels in Experiment I controls dropped one full µg percent during pregnancy, T_4 levels will not be identical at any two sample dates during pregnancy.

No depletion in T_4 was seen in the two highest PCB groups during the usual gestation period (Figures 1 and 4) since they were probably not pregnant or at least had begun reabsorbing their fetuses. Fetal death may result from fetotoxicity due to PCB's or from uteri unprimed for receiving and supporting an embryo. Hypothyroidism as was reported by Soliman and Reineke (1954) is closely associated with fetal loss, abortion and impaired reproduction. Thyroxine is known to be necessary for the preparation and maintenance of receptive uteri; therefore, mink on high PCB diets which were relatively hypothyroid during reproduction may have had insufficiently prepared uteri even though they had significantly greater thyroid activity than the controls during the rest of the year.

Additionally, several months of hyperthyrodism in the two high PCB groups may have led to a severe depletion in fat reserves. High BMR's stimulated by T_4 were not measured but may have contributed to a reduced availability of nutrients for the fetus. The energy depletion stress may have further compounded the T_4 and PCB factors in reducing reproduction to zero in the two highest PCB groups.

Excess thyroxine secretion which occurred (Figures 1 and 3) throughout autumn and winter in the two highest PCB groups had by February saturated the T_4 carriers far in excess of the normal TBG-capacity by a factor of 3 times (Table 6). Binding to prealbumin and albumins is the most probable explanation for the excess bound T_4. Through pregnancy and lactation in April and May the saturation index of the 5 ppm PCB group remains higher than the control but estrus, pregnancy and lactation has apparently stimulated a higher concentration of TBG since the TBG capacity is larger in the controls at that time. Dowling et al., 1956a; Braverman et al., 1967; and Zaninovich et al., 1966, have shown that estrogen increases TBG-capacity similar to these results (Table 8).

The stimulation of adrenal glands which was observed in the 5 ppm PCB (Table 8) group, could result from two sources. It may be due to the suppression of the negative feedback mechanism for adrenal steroids but more likely it results from adrenal gland stimulation by thyroxine.

Wallach and Reineke (1949) reported that exogenous thyroxine in rats increased adrenal weight and concurrently caused changes in adrenal ascorbic acid content. These are sensitive indicators for adrenal function, thereby providing evidence of a T_4-induced stimulating effect upon the adrenal gland. Iturri (1974) observed a PCB-induced adrenal weight increase in chickens which is similar to the one we have observed in mink.

Liver wt + Liver weight was increased by PCB's (Table 8) probably as a result of increased demand for detoxifying enzymes or as a result of metabolic stimulation by thyroxine or both. Grant et al., 1971; Rehfeld et al., 1971; and Lincer and Peakall, 1973, observed increases in liver weight in many other species which had been treated with PCB. Induction of liver enzymes by PCB's was also noted by Rhee and Plapp (1973).

Circulating thyroxine levels in male and female mink were essentially not different from each other throughout most of the year (Figure 8) but differed in different months. Female thyroxine secretion rates in January averaged 0.854 ± 0.175 µg/100 gm B.W./day which agrees extremely closely with that found in males by Reineke et al. (1960). They reported that TSR was equal to 0.95 µg/100 g B.W./day using the thyroxine substitution method of Reineke and Singh (1955). The close agreement of the two methods for estimating TSR is noteworthy considering the differences in the

thyroxine degradation method and the thyroxine substitution method (see Literature Review and Methods section for description of the two methods).

Distribution space for thyroxine was greater in the 5 ppm group than in the control groups (Figures 2-5). No edema was noticeable upon autopsy of the 5 ppm PCB group as reported in birds by Iturri (1974). It seems possible that the PCB's may open additional pools to thyroxine. For example, they may lower the blood brain barrier for T_4 or may expand the interstitial cell fluid space. Total extra thyroidal thyroxine (ETT) was correspondingly large in PCB treated animals (Figures 2-5). With more distribution space available, the total T_4 present within an animal would be predictably expanded.

Histology of the thyroids after 9 months on PCB's at 5 ppm revealed that thyroids were stimulated (Figure 12). They had numerous very large follicles, containing numerous vacuoles within the colloid and tall cuboidal epithelium surrounded the follicles. In contrast controls (Figure 11) had small follicles low cuboidal epithelium and few colloidal vacuoles.

Fig. 12 p. 102

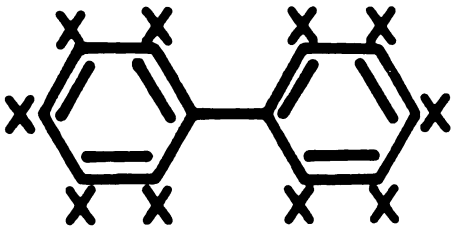
These morphological data further support the chemical data which suggest that stimulation of the thyroid produced high T_4 levels which were permitted to occur without slowing the thyroxine output rate. This evidence further supports

the hypothesis that the PCB's affected the CNS and suppressed the normal feedback system allowing high thyrotropin output in the face of high circulating thyroxine.

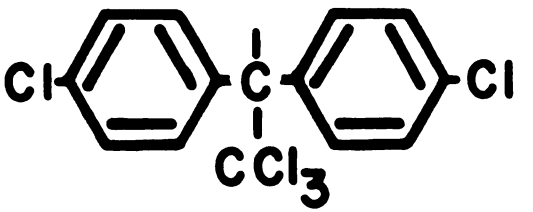
APPENDICES

APPENDIX A

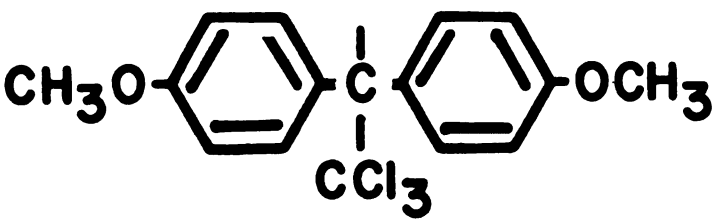
CHEMICAL STRUCTURE OF POLYCHLORINATED BIPHENYL (PCB)
AND RELATED COMPOUNDS



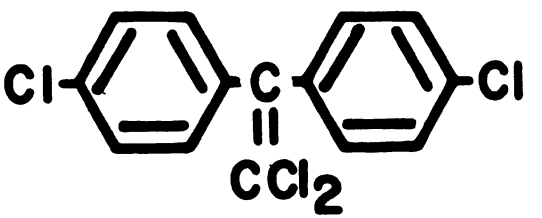
PCB



DDT



METHOXYCHLOR



DDE

APPENDIX B

BASIC MINK DIET

MINK DIET

FISH	30%
POULTRY	30%
TRIPE	15%
LIVER	5%
CEREAL	20%
	100%

APPENDIX C

TECHNIQUE FOR MEASURING BINDING CAPACITY OF TBG

A. Reagents and Solutions for TBG-Capacities

1. ^{125}I-L-thyroxine Solution

L-thyroxine ^{125}I in 50% aqueous propylene glycol (Amersham/Searle Corp., Arlington Heights, Illinois) was diluted in glass distilled water such that 0.05 ml of the diluted standard would yield 10,000 to 50,000 cpm. The contribution of this amount of labeled T_4 during the first 2 half-lives was less than 0.1 µg percent per sample.

2. Cold Thyroxine Solution

Ten mg of purified L-thyroxine was weighed to the nearest 0.1 mg and dissolved in glass distilled water with the aid of NaOH to dissolve the crystals. All glassware was siliconized to prevent T_4 adhesion. The cold T_4 was brought to a concentration of 0.02 µg/ml by serial volumetric dilution in glass distilled water at 25°C. A second concentration of 0.04 g/ml was also prepared to provide a more concentrated solution needed in the upper regions of the thyroxine binding curve.

3. Barbital Buffer (pH 8.6)

(a) N/10 HCl solution:

9.857 gm of concentrated reagent grade HCL (37% HCl) was weighed on a triple beam balance and was brought to 1000 ml volumetrically with glass distilled water.

(b) M/10 Sodium Barbital solution:

20.62 gm of powdered sodium barbital was weighed to the nearest 0.1 mg and diluted volumetrically to 1000 ml with glass distilled water.

(c) Preparation of Buffer:

129 ml of the HCl solution (a) was added from a burette into a 1000 ml volumetric flask and brought to volume with the sodium barbital solution (b). After thoroughly mixing with a Magna Stirrer the pH was checked using a Beckman pH meter with a Corning Semimicro electrode (Corning Glassworks, Medford, Mass.) probe. Any slight deviations from pH 8.6 were adjusted with HCl or NaOH.

B. Resin-impregnated Sponges and Polypropylene Tubes

Resin sponges with the capacity to absorb specifically free thyroxine (Abbott Radiopharmaceuticals, North Chicago, Illinois*) were cylindrical in shape and have the following dimensions: 1.1 cm O.D. x 1.95 cm. Dispersed within the polyurethane sponge is a finely divided ion exchange resin. Unbound thyroxine is quickly and quantitatively bound to the resin sponge while TBG-bound thyroxine is not. (IRA-400 anion-exchange resin may be substituted for the resin sponge.)

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*Thanks are due to Abbott Radiopharmaceutical for donating the sponges used for this procedure.

The polypropylene tubes had the following dimensions: 1.3 cm I.D. x 8.6 cm (Abbott Radiopharmaceuticals, North Chicago, Illinois) and adhered no thyroxine as non-siliconized glass tubes would. They were also reusable upon allowing the tracer to decay for a year or so and washing with Radiac Wash.

Computation of Thyroxine Concentration for the Binding Curve

A range of total exogenous T_4 levels was chosen from 12 to 34.6 µg percent in 1.0 µg increments. To compute the total endogenous plus exogenous levels, the serum T_4 level was added to each increment. To calculate µg added:

$$\begin{matrix}\text{µg percent cold} \\ \text{exogenous } T_4\end{matrix} = \begin{matrix}\text{µg } T_4\text{/ml concentration} \\ \text{of cold } T_4\end{matrix} \times \begin{matrix}\text{volume} \\ \text{cold } T_4\end{matrix} \times \frac{100}{0.05} \qquad \text{VIII}$$

The factor of 100/0.05 is used to transfer the units of cold T_4 into the same units as the 0.05 ml serum which was added, i.e., to µg percent.

$$\begin{matrix}\text{Total µg percent} \\ \text{added}\end{matrix} = \begin{matrix}\text{µg percent cold} \\ \text{exogenously}\end{matrix} + {}^{125}\text{I } T_4 + \begin{matrix}\text{Serum } T_4 \\ \text{µg percent}\end{matrix} \qquad \text{IX}$$

To calculate the amount of barbital buffer needed, 1.65 ml total volume minus volume cold T_4 = volume barbital buffer (always more than 2/3 of the total volume).

Procedural Sequences for Binding Curve

Into a series of polypropylene tubes was measured a sufficient quantity of barbital buffer (pH 8.6) to sum up with the volume of cold T_4 at a concentration of 0.02 µg/ml or at 0.04 µg/ml to a volume of 1.65 ml per tube. As the amount of cold thyroxine progressively increased in small increments, the volume of buffer decreased. Sequentially, to the barbital buffer was added 0.05 ml of ^{125}I labeled L-thyroxine from disposable microliter pipettes followed by a 15 second vortex mix.

Cold thyroxine was then added from a Hamilton 500 microliter syringe followed by another 15 seconds of vortex mixing. Lastly, serum was added to each sample and vortex-mixed for a minimum of 30 seconds. From previous experience, binding to TBG will be erratic unless the tubes are thoroughly mixed immediately after adding each component. Tubes were then immersed in a slowly shaking warm water bath at 37 $\pm$ 0.5°C and the reaction mixture was allowed to equilibrate for 1 hour, after which the resin impregnated sponges were added. Each sponge was gently depressed three times with a plastic plunger. Initial counts were taken using a gamma counter and scaler analyzer set to count at the ^{125}I peak. The tubes were incubated for 30 more minutes at 37 $\pm$ 0.5°C to allow the resin-impregnated sponges to absorb free thyroxine. Each tube was then immediately filled with distilled water and the sponges were washed and aspirated

with a plastic suction apparatus. The washing was repeated 3 times and each time the sponges were depressed gently with the suction apparatus and most of the liquid was removed. The TBG-bound thyroxine was removed with the washing, leaving only free thyroxine adhering to the resin impregnated sponges. A final count was then taken from each tube.

Calculations for Thyroxine Binding Curve

For each level of cold exogenous thyroxine added to a tube, µg percent T_4 bound to protein is calculated from the following equations:

$$\text{µg percent bound to resin sponge} = \frac{(\text{FCPM} - \text{Background cpm})}{\text{ICPM} - \text{Background cpm}} \times$$

$$(\text{correction factor A}) \qquad \text{X}$$

where the correction Factor A =

$$\frac{\text{Blank (serum free) ICPM} - \text{Background cpm}}{\text{Blank (serum free) FCPM} - \text{Background cpm}} =$$

Correction factor for free thyroxine not bound to resin impregnated sponge. XI

(correction factor A was usually slightly less than 1.00)

$$\text{µg percent } T_4 \text{ bound to protein} = \text{1-percent µg bound to resin sponge} \times \text{Total µg percent cold } T_4 \qquad \text{XII}$$

When µg percent T_4 bound to protein is plotted against total T_4 used the binding curve in Figure 9 results. From

the flat plateau portion of the binding curve the amount of T_4 necessary to saturate the TBG binding sites can be read. T_4 values above the plateau represent nonspecific binding, probably to albumin or other proteins. At very high T_4 concentrations, two criteria of the specificity of this method are violated. The dilution factor is less than 30-35 and the capacity of the barbital buffer (pH 8.6) to suppress the normally feeble binding to protein is exceeded at very high T_4 concentrations. Therefore, a level of total T_4 about midway through the plateau will provide sufficient thyroxine to saturate most, if not all, of the sites.

Procedural Sequences for TBG Capacity

Into each of 3 polypropylene tubes was measured a sufficient quantity of barbital buffer (pH 8.6) to sum with the volume of cold T_4 at a concentration of 0.02 µg/ml to a volume of 1.65 ml per tube and a dilution factor for serum of 30-35. Two of the tubes were designated as duplicates containing serum and the third was a serum-free blank. Barbital buffer was added with a Hamilton microliter syringe. To each tube was added 0.05 ml ^{125}I-L-thyroxine using disposable microliter pipettes followed by 15 seconds of vortex mixing. Next in sequence, unlabeled thyroxine at 0.02 µg/ml was added to each tube and vortex-mixed for 15 seconds. Lastly, serum was added to the two duplicates but none was added to the blank. Each tube was then vortex-mixed for 30

seconds to ensure equal distribution of each component. Tubes were immersed in a slowly shaking water bath at 37° ± 0.5°C for 1 hour and were then treated from this step in the same fashion as those tubes described in the binding curve method.

Calculation of T_4 Concentration Necessary for Determining TBG Capacity

From the plateau region of the binding curve, a concentration of total T_4 was chosen which would saturate most, if not all, of the available thyroxine binding sites but not exceed the capacity of the barbital buffering system and the dilution factor of 30-35 to inhibit nonspecific protein binding.

(1) Amount (µg percent) of cold T_4 needed =

Total T_4, µg percent (exogenous + endogenous) - serum T_4, µg percent - ^{125}I-T_4 XIII

(2) Volume cold T_4 needed (ml) =

$$\frac{\text{Amount cold } T_4 \text{ needed (1)}}{\text{Concentration of cold } T_4, \text{ µg/ml}} \quad \text{XIV}$$

(3) Buffer volume = 1.65 ml total volume - (of buffer + cold T_4)

ml cold T_4 XV

Calculations for TBG-Capacity and Saturation Index

$$\frac{\text{FCPM - background cpm}}{\text{ICPM - background cpm}} \times \text{correction factor B} = \text{µg percent bound to resin sponge} \quad \text{XVI}$$

where the correction factor B =

$$\frac{\text{Blank (serum free) ICPM - background cpm}}{\text{Blank (serum free) FCPM - background cpm}} =$$

correction factor for free thyroxine not adhered to resin impregnated sponge.

$$\mu g \text{ percent } T_4 \text{ bound to TBG} = \text{1-}\mu g \text{ percent bound to resin sponge} \times \frac{\text{Total } \mu g \text{ percent used}}{\text{Serum thyroxine}} \quad \text{XVII}$$

$$\text{Saturation Index (SI)} = \frac{\text{Serum thyroxine}}{\mu g \text{ percent/}\mu g \text{ percent } T_4 \text{ bound to TBG}} \quad \text{XVIII}$$

Correction for Thyroxine Not Bound by Resin Sponges

Correction factors A and B in the Calculation Section were determined by preparing blank tubes to contain precisely identical labeled and unlabeled thyroxine as do the duplicates but contain no serum. Since there was no TBG in the blanks, any observable difference between the blank ICPM and the blank FCPM was a result of the amount of free thyroxine not taken up by the sponges. The fractional correction of ICPM/FCPM will correct for this usually small difference. The correction fraction is used to correct the sample FCPM, which assumably had also left unbound a similar quantity of free T_4.

APPENDIX D

THYROXINE LEVEL (μG/100 ML PLASMA) OF FEMALE MINK RECEIVING FOUR TREATMENTS (5 PPM PCB, 2 PPM PCB, 0.5 PPM PCB AND A CONTROL) FROM EXPERIMENT I

MINK EXPERIMENT 7309

Mink No.	Sept. 9-19-73	Oct. 10-17-73	Nov. 11-14-73	Dec. 12-12-73	Jan. 1-9-74	Feb. 2-7-74	Mar. 3-6-74	Apr. 4-3-74	May 5-8-74	June 6-1-74
Control										
A 512	2.19	3.16	3.44	1.13	1.78	1.32	2.33	3.03	2.51	1.88
A 294	2.07	3.60	2.93	1.18	2.17	1.93	2.84	3.89	3.09	2.96
A 394	1.74	2.96	3.03	2.16	1.87	1.84	2.14	3.14	2.67	2.08
A 404	2.15	2.94	2.82	1.93	2.00	2.31	2.94	3.56	1.92	2.54
A1240	2.40	3.24	2.86	1.14	1.37	1.89	1.81	2.67	1.26	3.59
A 372	2.60	3.82	1.25	1.38	1.70	1.84	2.54	3.48	2.60	3.71
A 300	1.76	3.11	-	2.36	2.00	2.12	1.82	2.07	1.62	2.93
$\bar{X}$	2.16	3.26	2.72	1.61	1.84	1.89	2.34	3.12	2.18	2.77
S.E.	0.10	0.12	0.75	0.19	0.25	0.11	0.17	0.23	0.23	0.23
0.5 ppm PCB										
A 182	1.98	2.38	-	2.94	1.12	2.54	2.88	3.84	1.98	4.49
A 290	2.26	1.86	3.42	2.86	-	1.74	2.18	4.36	1.75	-
A 70	2.00	2.46	2.17	2.60	2.53	1.98	3.31	3.11	-	3.06
A 302	3.11	3.33	1.82	2.54	3.13	3.09	4.64	2.86	1.87	2.38
A 370	-	2.40	3.46	1.75	3.85	2.23	4.09	2.30	2.42	2.97
A 660	2.85	2.90	2.96	3.73	2.52	3.48	2.95	4.24	2.68	3.97
A 510	1.60	3.37	3.31	2.27	2.78	1.96	2.34	3.79	2.27	3.20
A 312	1.72	3.35	2.91	3.62	2.43	1.67	3.22	3.28	2.61	3.28
$\bar{X}$	2.21	2.75	2.87	2.79	2.62	2.34	3.20	3.47	2.21	3.34
S.E.	0.56	0.56	0.25	0.20	0.31	0.65	0.29	0.25	0.14	0.26

continued

APPENDIX D--MINK EXPERIMENT 7309--continued

Minl No.	Sept. 9-19-73	Oct. 10-17-73	Nov. 11-14-73	Dec. 12-12-73	Jan. 1-9-74	Feb. 2-7-74	Mar. 3-6-74	Apr. 4-3-74	May 5-8-74	June 6-1-74
2 ppm PCB										
A 190	2.33	2.82	2.43	2.09	1.62	-	-	-	-	-
A 214	1.28	3.29	2.16	3.37	1.12	2.88	1.69	2.86	2.59	3.94
A 406	2.84	2.32	2.33	2.40	1.96	2.48	3.53	2.85	2.13	3.13
A 380	1.68	2.12	2.22	2.94	2.96	2.56	-	3.60	4.08	2.72
A 352	1.86	2.26	2.07	2.27	2.16	1.95	2.70	1.91	1.72	4.10
A 32	2.37	3.27	2.65	2.48	2.38	3.52	2.74	2.67	1.36	3.28
A 304	1.74	2.28	3.12	3.31	3.86	3.14	3.07	1.81	3.35	2.97
A 306	2.47	3.82	2.36	3.12	2.70	3.51	4.16	2.86	4.22	3.65
$\bar{X}$	2.07	2.77	2.42	2.74	2.34	2.86	2.98	2.65	2.78	3.40
S.E.	0.18	0.22	0.11	0.17	0.30	0.21	0.84	0.23	0.42	0.19
5 ppm PCB										
A 820	1.75	3.73	3.41	3.04	2.07	4.09	2.09	2.15	2.07	3.48
A 292	1.82	3.78	4.48	-	3.44	2.57	1.31	2.84	1.15	3.22
A 308	2.19	4.07	4.06	3.18	2.86	3.09	2.75	-	2.12	3.53
A 2	2.44	2.43	-	3.32	2.32	4.46	2.57	1.67	-	2.84
A 184	2.27	3.09	4.27	2.64	2.90	3.30	2.34	2.47	2.34	2.75
A 472	2.40	2.75	3.04	-	2.39	3.74	2.96	2.37	2.96	3.10
A 192	2.33	2.90	3.62	2.60	3.10	4.11	2.75	3.50	4.28	3.94
A 450	1.56	3.11	3.44	2.44	2.92	2.48	2.07	2.95	2.68	3.31
$\bar{X}$	2.09	3.23	3.76	2.87	2.73	3.48	2.36	2.56	2.51	3.27
S.E.	0.11	0.20	0.19	0.14	0.18	0.26	0.18	0.22	0.36	0.13

APPENDIX E

THYROID PARAMETERS OF FEMALE MINK FROM EXPERIMENT II

CONTROLS JANUARY (1-2-74)

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% ose at ime TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coeffi-cient of Corre-lation
A 42	885	-0.0436	0.599	0.0982	6.90	166	18.75	1.87	0.351	0.827	.994
A 812	885	0.0764	0.906	0.1695	3.98	110	12.42	1.86	0.231	0.939	.995
A 692	885	-0.0496	0.799	0.1114	6.07	125	14.12	0.94	0.133	0.356	.996
A 892	885	-0.0477	0.722	0.1074	6.30	138	15.59	1.78	0.277	0.714	.999
696	885	-0.0441	0.375	0.0995	6.81	266	30.06	2.00	0.601	1.436	.996
$\bar{X}$		0.0523	0.680	0.1172	6.00	161	18.19	1.69	0.319	0.854	
S.E.		0.0061	0.091	0.0133	0.54	27.8	3.14	0.19	0.078	0.175	

continued

APPENDIX E--continued--FEBRUARY (2-22-74) CONTROLS

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% Dose at Time TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coefficient of Correlation
Q2376	1120	-0.0477	0.832	0.1074	6.29	120.19	10.73	1.04	0.111	0.287	0.999
T 160	720	-0.0528	0.952	0.1184	5.69	105.04	14.59	1.73	0.252	0.717	0.997
T 440	920	-0.0479	0.818	0.1076	6.27	122.25	13.29	1.84	0.244	0.632	0.995
A 416	680	-0.0592	1.030	0.1323	5.08	97.08	14.28	0.98	0.139	0.444	0.999
T 240	920	-0.0401	0.930	0.0904	7.50	107.53	11.69	1.43	0.167	0.363	0.977
A 620	900	-0.0529	0.795	0.1188	5.68	125.79	13.98	0.69	0.096	0.275	0.995
R1030	900	-0.0449	0.965	0.1013	6.69	103.63	11.51	1.22	0.140	0.341	0.997
$\bar{X}$	880	-0.0460	0.903	0.1109	6.17	111.64	12.86	1.28	0.164	0.437	
S.E.	55	0.0035	0.033	0.0050	0.29	4.14	0.58	0.15	0.023	0.065	

continued

APPENDIX E--continued--FEBRUARY (2-22-74) 5 PPM PCB

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% Dose at Time TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coefficient of Correlation
A 120	1010	-0.0444	0.487	0.0999	6.78	205.33	20.33	3.67	0.746	1.79	0.994
A 620	1170	-0.0528	0.616	0.1184	5.69	162.34	13.87	4.34	0.602	1.71	0.996
A 602	890	-0.0507	0.576	0.1138	5.93	173.61	19.51	3.59	0.700	1.91	0.995
A 690	900	-0.0600	0.592	0.1342	5.01	177.94	19.77	3.13	0.619	1.99	0.994
A 64	800	-0.0787	0.780	0.1744	3.82	128.20	16.03	4.23	0.678	2.83	0.999
A1244	900	-0.0648	0.595	0.1444	4.64	168.06	18.67	3.92	0.731	2.53	0.996
A 580	750	-0.0641	0.438	0.1431	4.69	228.31	30.44	3.32	1.010	3.47	0.997
A 700	780	-0.0636	0.591	0.1420	4.72	169.20	21.69	3.37	0.252	2.49	0.999
$\bar{X}$	900	-0.0599	0.581	0.1332	5.16	176.62	22.04	3.69	0.667	2.34	
S.E.	137	0.0037	0.035	0.0082	0.32	10.50	1.72	0.15	0.074	0.21	

continued

APPENDIX E--continued--APRIL (4-12-74) CONTROLS

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% Dose at Time TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coefficient of Correlation
Q2376	1220	-0.0494	1.130	0.1109	6.09	88.50	7.25	0.98	0.071	0.189	0.989
T 160	880	-0.0376	1.145	0.0849	8.00	87.34	9.92	1.65	0.163	0.334	0.997
T 440	1060	-0.0483	1.060	0.1087	6.22	94.34	8.90	1.29	0.114	0.299	0.990
A 416	920	-0.0485	1.180	0.1089	6.19	84.75	9.21	1.11	0.102	0.267	0.999
T 240	890	-0.0314	1.167	0.0711	9.58	85.69	8.74	1.05	0.091	0.156	0.991
A 620	1000	-0.0451	1.080	0.1017	6.66	92.59	9.26	1.82	0.168	0.411	0.989
R1030	920	-0.0412	1.166	0.0929	7.30	85.76	9.32	1.83	0.170	0.380	0.992
$\bar{X}$	997	-0.0431	1.132	0.0970	7.150	88.42	8.943	1.39	0.125	0.291	
S.E.	43	0.0025	0.017	0.0056	0.483	1.39	0.315	0.13	0.015	0.035	

continued

APPENDIX E--continued--APRIL (4-12-74) 5 PPM PCB

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% Dose at Time TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coefficient of Correlation
A 120	760	-0.0802	1.176	0.1774	3.75	85.03	11.18	2.25	0.252	1.076	0.996
A 620	860	-0.0586	1.147	0.1310	5.14	87.18	10.13	2.98	0.302	0.949	0.979
A 602	740	-0.0734	1.080	0.1631	4.10	92.59	12.51	2.13	0.266	1.043	0.984
A 690	880	-0.0623	1.030	0.1390	4.83	97.09	11.03	1.89	.208	0.696	0.980
A 64	740	-0.0701	0.993	0.0920	7.38	100.70	13.25	1.80	.293	0.641	0.917
A1244	760	-0.0408	0.993	0.0920	7.38	100.70	13.25	2.21	.793	0.647	0.917
A 580	640	-0.0644	1.030	0.1438	4.66	97.09	15.17	2.68	0.406	0.608	0.994
A 700	780	-0.0642	1.130	0.1431	4.69	88.50	11.34	3.74	0.424	1.454	0.999
$\bar{X}$	770	-0.0642	1.090	0.1352	4.854	92.01	12.061	2.46	0.296	0.909	
S.E.	26	-0.0041	0.023	0.0107	0.392	2.01	0.552	0.229	0.028	0.001	

APPENDIX E--continued--MAY (5-16-74) 5 PPM PCB LACTATING CONTROLS AND NON-LACTATING CONTROLS

Mink Number	Body Weight	Log n Slope	Percent Dose/ml at Time Zero	Rate Constant K	$t_{\frac{1}{2}}$	100% Dose at Time TDS (ml)	TDS/100 gm Body Weight	T_4 µg/ 100 ml	ETT	TSR	Coeffi-cient of Corre-lation
PCB											
A1244	795	-0.0403	0.414	0.0909	7.46	241	30.31	2.85	0.863	1.880	0.989
A 64	875	-0.0219	0.285	0.0498		351	40.01	3.58	1.432	1.710	0.968
A 690	1050	-0.0456	0.372	0.1026	6.60	269	25.62	3.30	0.845	2.080	0.939
A 602	890	-0.0490	0.451	0.1100	6.13	222	24.94	3.81	0.950	2.510	0.894
A 620	945	-0.0441	0.413	0.0995	6.81	242	25.61	3.05	0.781	1.870	0.997
A 580	700	-0.0429	0.597	0.0966	7.02	161	23.85	1.99	0.474	1.100	0.816
A 120	930	-0.0609	0.462	0.1302	4.94	216	23.23	3.26	0.757	2.370	0.877
$\bar{X}$	8847	-0.0435	0.427	0.0971	6.49	244	27.65	3.12	0.872	1.930	
S.E.	42	-0.0044	0.035	0.0092	0.35	21	0.223	0.109	0.175	0.175	
Lactating Controls											
T 240	1005	-0.0421	0.419	0.0949	7.14	239	33.42	2.26	0.755	1.720	0.998
Q2376	1205	-0.0396	0.390	0.0896	7.59	256	21.28	1.63	0.346	0.740	0.836
T 160	920	-0.0360	0.419	0.0817	8.35	238	25.94	2.12	0.549	1.080	0.956
A 416	945	-0.0358	0.393	0.0810	8.41	254	26.93	2.13	0.637	1.240	0.944
P2044	840	-0.0381	0.346	0.0860	7.90	289	34.40	2.10	0.722	1.490	0.966
$\bar{X}$	983	0.0383	0.393	0.0866	7.88	255	28.39	2.05	0.602	1.250	
S.E.	61	0.0010	0.013	0.0024	0.23	9	2.45	0.108	0.073	0.160	
Non-lactating Controls											
R 620	1110	-0.0316	0.374	0.0716	9.52	261	24.09	2.59	0.623	1.070	0.868
R1030	810	-0.0339	0.441	0.0764	8.86	227	27.99	2.12	0.593	1.100	0.995
T 440	1240	-0.0383	0.428	0.0867	7.84	233	18.84	2.17	0.408	0.852	0.888
$\bar{X}$	1053	0.0346	0.414	0.1784	8.740	242	23.64	2.29	0.542	1.007	
S.E.	127	0.0017	0.020	0.0044	0.489	12	2.65	0.149	0.067	0.073	

APPENDIX F

THYROXINE LEVELS (µG/100 ML PLASMA) OF MALE AND FEMALE MINK FROM EXPERIMENT III

FEMALE MINK

	Aug. 8-22-72	Dec. 12-30-74	Feb. 2-21-73	Mar. 3-24-73	Apr. 4-14-73	May	July
T 488	2.03	2.07	4.08	1.73	-	2.58	2.28
TP200	0.99	1.86	2.00	1.65	-	4.26	4.12
T 226	1.74	1.98	3.44	1.89	-	2.66	2.32
T 52	0.70	1.95	1.97	2.67	1.37	1.44	2.36
T 162	1.37	1.10	-	1.07	0.75	2.36	2.54
T 180	0.80	2.32	-	2.36	-	-	2.43
TP1094	1.68	2.69	1.06	2.11	-	3.41	2.04
TP 86	0.78	1.66	2.45	0.44	1.19	2.10	2.34
T1042	1.75	2.57	2.46	1.88	-	1.51	2.15
TP214	1.00	1.52	-	-	-	2.12	2.30
T 400	2.03	2.15	1.31	1.56	-	2.00	3.42
TP 10	1.37	2.30	2.46	1.45	-	1.42	2.07
TP532	1.58	0.87	2.43	1.83	-	1.33	1.61
TP1068	1.74	2.38	2.66	1.61	1.65	1.50	3.20
T 270	2.03	2.26	2.22	1.24	0.86	1.10	2.65
n =	15	15	12	12	5	14	15
$\bar{X}$	1.43	1.97	2.39	1.67	1.16	2.12	2.52
S.E.	0.10	1.10	0.28	0.14	0.14	0.22	0.14

APPENDIX F--continued

MALE MINK

	Aug. 8-22-72	Dec. 12-30-72	Feb. 2-27-73	Mar. 3-24-73	Apr. 4-14-73	May	July
TP171	1.01	1.43	-	-	-	-	2.42
TP881	1.44	0.46	2.02	2.10	0.42	0.95	1.81
T 211	2.00	0.71	3.15	0.55	2.34	2.37	1.23
T 213	1.67	1.70	1.44	1.51	1.72	1.035	1.51
TP339	0.93	2.38	1.25	-	-	-	-
T 305	2.41	1.04	-	-	-	-	-
T 21	-	-	4.18	0.96	1.54	-	-
TP310	-	-	-	1.47	-	2.95	2.18
TP331	-	-	-	0.45	0.96	1.39	1.63
TP1061	-	-	-	-	-	-	1.37
n =	6	6	5	6	5	5	6
$\bar{X}$	1.57	1.28	2.40	1.17	1.39	1.93	1.72
S.E.	0.22	0.28	0.51	0.24	0.31	0.43	0.17

APPENDIX G

FEMALE MINK BODY WEIGHT (GRAMS) OF ANIMALS IN EXPERIMENT I

	Sept. 9-19-73	Oct. 10-17-73	Nov. 11-12-73	Dec. 12-12-73	Jan. 1-9-74	Feb. 2-7-74	Mar. 3-6-74	Apr. 4-3-74	May 5-8-74	May/June 5-20-74
Control										
$\bar{X}$	920	1060	1040	1032	885	938	878	912	844	846
S.E.	23	35	40	35	37	36	32	29	48	26
0.5 ppm PCB										
$\bar{X}$	947	1028	1055	1032	988	937	903	882	931	937
S.E.	21	33	124	45	31	39	31	25	31	20
2 ppm PCB										
$\bar{X}$	976	1066	950	1008	917	902	881	903	885	825
S.E.	44	48	39	40	47	50	53	45	51	49
5 ppm PCB										
$\bar{X}$	929	1047	1021	963	924	879	819	771	925	791
S.E.	32	54	56	44	37	40	35	27	35	39

APPENDIX H

Formula used in the Scheffé F ratio

For comparison of the "A" effect between treatments:

$$F = \frac{[\underline{C}_j\ (A) + (C'_j\ A_2)]^2}{MS_{\text{Subj. W. Groups}}\ \frac{(C_j)^2}{nq} + \frac{(C'_j)^2}{nq}}$$

Critical Value = $(K - 1)\quad (F = 0.05;\ V_1,\ V_2)$

When $V_1 = (p - 1)\ V_2 - P(n-1)$ and K = Number of means compared

For comparison of the "B" effect between times:

$$F = \frac{[C_j\ (B_1) + (C'_j\ B_2)]^2}{MS_{\text{BX Subj. W. Groups}}\ \frac{(C_j)^2}{np} + \frac{(C'_j)^2}{np}}$$

= Test Statistic

Critical Value = $(K - 1)\quad F = 0.05;\ V_1,\ V_2$

When $V_1 = (q - 1)$, $V_2 = P\ (n - 1)\ (q - 1)$ and

K = number of mean comparisons.

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