This is to certify that the

thesis entitled

Effects of 3,3',4,4'-tetrachlorobiphenyl on reproductive performance and gamete fertilizing ability in mice

presented by

Jeng-Fang Huang

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

Major professor

Date 8/25/95

EFFECTS OF 3,3',4,4'-TETRACHLOROBIPHENYL ON REPRODUCTIVE PERFORMANCE AND GAMETE FERTILIZING ABILITY IN MICE

BY

Jeng-Fang Huang

A THESIS

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

MASTER OF SCIENCE

Department of Animal Science

1995

Dr. Karen Chou

ABSTRACT

EFFECTS OF 3,3',4,4'-TETRACHLOROBIPHENYL ON REPRODUCTIVE PERFORMANCE AND GAMETE FERTILIZING ABILITY IN MICE

By

Jeng-Fang Huang

The objectives of this study were to investigate the reproductive effects of 3,3',4,4'-tetrachlorobiphenyl (TCB) on mature female mice (F-0) and the effects of perinatal and continuous TCB exposure on reproductive performance and gamete fertilizing ability of the offspring (F-1). F-0 mice were treated with 0, 3 ppm, and 30 ppm TCB two weeks before breeding. Treatments continued through mating, gestation, and lactation. F-1 mice were given the same diet as their dams throughout this study.

After 2 weeks of treatment, both fecundity and offspring survival in F-0 females decreased in the 30 ppm-treated group. Egg fertilizing ability *in vitro* in F-1 females decreased in the 3 ppm and 30 ppm groups. This decrease was associated with an increase in degenerated eggs. All offspring from 3 ppm and 30 ppm-treated F-1 females died within 4 days of birth. Increased testis weights were observed in the 30 ppm-treated F-1 weanlings, but not in pubertal and mature mice. Results of sperm motion analysis for F-1 males were the same among all treatment groups, except for decreased sperm motility observed in 9-week-old 30 ppm-treated males 2.5 hours after collection. The percentage of eggs fertilized *in vitro* by sperm from 19-week-old 30 ppm-treated F-1 males decreased. Increased body weights were observed in offspring of 17-week-old 30 ppm-treated F-1 males. Reproductive performance and gamete fertilizing ability were impaired by TCB exposure.

ACKNOWLEDGMENTS

The completion of this thesis would not have been possible without the help of many individuals. I am very grateful for those who have helped to design or conduct the research.

I would like to express my deepest gratitude to Dr. Karen Chou for not only her advice in scientific thinking, but also her financial support. It has been a privilege to work with such a distinguished and respected mentor. I greatly appreciate Dr. Thomas Voice and his group for their help in the feed and tissue analysis. Drs. Robert Cook and Steven Bursian deserve many thanks for their enthusiastic guidance in interpreting the data.

Special thanks are due to Amy Barber for her help in taking care of the animals. I appreciated the learning environment provided by my fellow graduate students Li Chen, James Kuo, Debbie Powell, Rochelle Inglis, Chang-Yi Lin, Barbara Salem, and Kathy Li. I am very thankful to my wife Shirley who gave me help whenever I needed it. I am also indebted to my son Ryan and my parents who taught me how to be tough and happy in my life.

This study was supported by NIEHS Superfund Grant P42 ES04911.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALH Dis.	amplitude of lateral head displacement
BW	body weight
4-CB	2,2',4,4'-tetrachlorobiphenyl
6-CB	2,2',4,4',5,5'-hexachlorobiphenyl
DHT	dihydrotestosterone
HCB	3,3',4,4',5,5'-hexachlorobiphenyl
HCG	human chorionic gonadotropin
i.p.	intraperitoneal
3MC	3-methylcholanthrene
PB	phenobarbital
PCBs	polychlorinated biphenyls
P_5CB	3,3',4,4',5-pentachlorobiphenyl
PMSG	pregnant mare's serum gonadotropin
TCB	3,3',4,4'-tetrachlorobiphenyl
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	toxic equivalent factor
TEq	toxic equivalent
VLDL	very low density lipoprotein

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a family of congeners with different numbers of chlorines binding covalently to biphenyl rings. Chlorination of biphenyl rings can produce 209 possible chlorobiphenyls, each substituted with 1-10 chlorine atoms (Sawhney, 1986). This group of chemicals was first synthesized by Schmidt and Schulz in 1881 and was commercially produced beginning in 1929 (Tanabe, 1988). PCBs have been frequently used in industry as heat transfer fluids, hydraulic fluids, lubricants, transformers, capacitors, plasticizers, and petroleum additives due to their chemical and physical stability and versatility (Richardson and Waid, 1982; Sawhney, 1986; Safe *et al.*, 1987). Polychlorinated biphenyls are resistant to acids and bases, compatible with organic materials, resistant to oxidation/reduction, nonflammable, nonconducting, and heat-resistant. However, improper disposal practices and accidental leakages from industrial facilities have led to environmental contamination (Tanabe, 1988; Safe, 1994). In addition to the flow of ground and surface water, the dynamic activity of the atmosphere accounts for much movement in the ecosphere (Atlas *et al.*, 1986; Hansen, 1987). PCBs have, therefore, been detected in the atmosphere of polar areas (Atlas *et al.*, 1986).

Exposure to PCBs has been linked to public health risks on numerous occasions.

Accidental human poisoning from PCB contaminated rice oil occurred in Japan in 1968, exposing over 1000 individuals (Rogan, 1982; Kashimoto and Miyata, 1986). Over 20 deaths were reported among these individuals within 5 years of contamination. A similar poisoning outbreak occurred in Taiwan in 1979 (Chen *et al.*, 1981; Rogan *et al.*, 1988). Within 4 years of the accident, 24 deaths were reported among 2060 poisoned patients (Chen and Hsu, 1989). Approximately half of the deaths were from hepatoma, liver cirrhosis, or other liver diseases accompanied by hepatomegaly (Chen and Hsu, 1989). Other clinical symptoms observed included mucocutaneous pigmentation, acneform eruptions, increased eye discharge, swelling of the upper eyelids, abnormal menstrual cycles, dark-brown pigmented babies, and stillbirths (Hirayama, 1976; Kikuchi and Masuda, 1976; Chen and Hsu, 1989).

Toxic responses to PCBs in laboratory animals have been extensively reviewed (Fishbein, 1974; Hansen, 1987; Parkinson and Safe, 1987). Hepatomegaly is a common syndrome observed in PCB-treated animals (Villeneuve and Grant, 1971; Sanders *et al.*, 1974; Clarke *et al.*, 1984). Other types of liver damage include centralobular degeneration, centralobular liver cell atrophy, focal necrosis, cytoplasmic vacuolization, and bile-duct proliferation (Curley *et al.*, 1973; Burse *et al.*, 1974). In addition, wasting syndrome (Garthoff *et al.*, 1977), hepatic and gastric intestinal hyperplasia (Ward, 1985), lymphoid involution (McConnell, 1985), porphyria (Safe, 1984), skin disorders (Barsotti *et al.*, 1976), carcinogenesis (Hansen, 1987), teratogenesis (Simmons *et al.*, 1984), and endocrine and reproductive dysfunction (Allen *et al.*, 1979; Golub *et al.*, 1991) have all been observed.

Reproductive disturbances caused by PCB exposure have been identified in many species. Female dysfunctions include increased and reduced germ cell numbers in mice (Ronnback, 1991; Ronnback and de Rooij, 1994), low frequencies of implanted embryos and embryotoxicity in mice (Orberg and Kihlstrom, 1973; d'Argy *et al.*, 1987), decreased litter sizes in rats (Brezner *et al.*, 1984), and prolonged estrous cycles in both mice and rats (Orberg and Kihlstrom, 1973; Brezner *et al.*, 1984). High incidences of abortion were also evident in Aroclor 1248 exposed non-human primates (Barsotti *et al.*, 1976). Linzey (1988) reported reduced ovary, uterus, and body weights in F-1 mice exposed to Aroclor 1254 *in utero* and through lactation. Male reproductive abnormalities have also been reported. Increased relative testis weights (mg testis/100 g bw) have been observed in mice exposed to 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB) perinatally (Johansson, 1987). Sanders *et al.* (1977) reported reduced testicular spermatozoa concentrations in Aroclor 1254-treated mice. Decreased number and percentage of normal fertilized eggs and eggs at the 2-cell, 4-cell, and blastocyst stages were observed in non-treated females mated to males exposed to Aroclor 1254 through milk on days 1, 3, 5, 7, and 9 postpartum (Sager *et al.*, 1991). Altered hepatic microsomal testosterone hydroxylase activities were observed in adult male and female rats which were administered Aroclor 1254 postnatally (Haake-McMillan and Safe, 1991).

PCB congeners vary in toxicity depending on their chemical structure. Quantitative structure-activity relationships (QSARs) have been investigated both *in vivo* and *in vitro* (Leece *et al.*, 1985). *In vivo* QSARs have been determined by ED50 values for cytochrome P-448-dependent monooxygenases induction, body weight loss, as well

as thymic atrophy in immature rats. *In vitro* QSARs have been developed from their potencies as inducers of cytochrome P-448-dependent monooxygenases and relative A*h* receptor binding affinities in rat hepatoma H-4-II-E cells. A high rank order correlation between *in vivo* QSARs data and the *in vitro* QSARs has been observed (Leece *et al.*, 1985).

The three coplanar congeners, 3,3',4,4'-tetrachlorobiphenyl (TCB), 3,3',4,4',5-pentachlorobiphenyl (P_5CB), and 3,3',4,4',5,5'-hexachlorobiphenyl (HCB), are approximate isostereomers of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and are capable of eliciting similar toxicity (Safe, 1984). Based on the hepatic microsomal monooxygenases inducing potency or A*h* receptor binding affinity, TCB, P_5CB, and HCB are classified as Group I, where other PCB congeners are classified as Group II. Group I congeners are more potent than Group II congeners as inducers of cytochrome P-448-dependent monooxygenases activity in cultured rat hepatoma H-4-II-E cells (Sawyer and Safe, 1982). On the basis of A*h* receptor binding affinity, Group I congeners are more competitive than Group II congeners in displacing TCDD from rat liver A*h* receptors (Bandiera *et al.*, 1982; Parkinson and Safe, 1987). Many of the adverse effects elicited by PCBs appear to be dependent on the presence of A*h* receptors in target organs (Poland and Knutson, 1982; Nebert, 1989). The A*h* receptor-ligand complex regulates the transcription of two P450I genes, *Cyp1a1* and *Cyp1a2* (Nebert, 1989). The receptor appears to have a recognition site to which PCB isosteric compounds with a configuration similar to TCDD can bind (Poland and knutson, 1982). High reproductive and developmental toxicities of these approximate isostereomers of

TCDD-TCB, P_5CB, and HCB are attributed, in part, to the presence of A*h*-receptor in embryonic tissue and *Cyp1a1* mRNA in ovarian cells (Dencker and Pratt, 1981; Safe, 1984; Nebert, 1989).

LITERATURE REVIEW

Physical and Chemical Properties of PCBs

Polychlorinated biphenyls (PCBs) have been identified in diverse environmental matrices worldwide. PCBs are produced by the chlorination of biphenyl rings and their names reflect the percentage of chlorine content. For example, Aroclor 1221, a commercial product produced by Monsanto Chemical Company (U.S.A.), contains 21% chlorine by weight. Similar commercial PCB mixtures have been produced by other manufacturers. These include the Clophens (Bayer, Germany), Phenoclors and Pyralenes (Prodelec, France), Fenclors (Caffaro, Italy), Kanechlors (Kanegafuchi, Japan), Soval (Sovol, U.S.S.R.), and Delor (Chemko, Czechoslovakia) (Safe, 1984; Safe, 1994). Because of their chemical stability, heat resistance, and miscibility with organic compounds, PCBs have been used as organic diluents, plasticizers, pesticide extenders, adhesives, dust-reducing agents, cutting oils, flame retardants, heat transfer fluids, dielectric fluids for transformers and capacitors, hydraulic lubricants, and sealants. Some of the uses of PCBs have resulted in their direct introduction into the environment, however, a significant portion of the environmental burden of these compounds has resulted from careless disposal practices, accidents, leakages, and mishandling at chemical waste disposal sites (Safe, 1994).

PCB congeners with zero or one *ortho* chlorine, two *para* chlorines, and at least

two *meta* chlorines can assume a coplanar conformation sterically similar to that of TCDD, therefore their toxicity is similar to TCDD. In contrast, PCB congeners with two or more *ortho* chlorines cannot assume a coplanar conformation and do not resemble TCDD in toxicity (Poland and Knutson, 1982; Safe, 1990). For those non-*ortho* and mono-*ortho* PCBs, dose dependent and structure dependent correlations were observed between aryl hydrocarbon hydroxylase (AHH) induction and several toxic responses such as thymic atrophy, reduced body weight gain, and teratogenicity in laboratory animals (Safe, 1990).

Absorption

Mammals can take up PCBs by three mechanisms (Shaw and Connell, 1990). They are:

1. absorption of PCBs from the atmosphere through the lungs.
2. absorption of PCBs from the atmosphere through the epidermis.
3. oral intake.

The atmospheric route through the lungs and epidermis is of little significance due to the low concentrations of PCBs in the atmosphere. Gastrointestinal absorption of commercial PCB mixtures and individual congeners has been investigated extensively in laboratory animals (Matthews and Anderson, 1975; Gage and Holm, 1976; Tanabe *et al.*, 1981). A complex mixture of Kanechlors 300, 400, 500, and 600 (1:1:1:1 by weight) in corn oil was administered orally to immature Wistar rats. The rats were dosed with 3 mg daily for 5 days. During this 5-day period, more than 84% of the total dose was absorbed from the gastrointestinal tract. Gas chromatograph-mass spectrometer analysis

of the PCBs in the fecal material demonstrated that the absorption efficiency of individual PCBs varied from 66%-96% (Tanabe *et al.*, 1981). The major structural determinants which govern absorption efficiencies in the intestinal wall are molecular size and lipophilic properties. It is generally expected that more small and highly lipophilic molecules will be more easily absorbed through the cell wall than large lipophobic molecules. However, the absorption efficiency of PCBs decreased as the number of chlorine atoms increased (Tanabe *et al.*, 1981). This suggests that the differences in lipophilic properties among individual chlorobiphenyls plays a minor role in absorption.

Distribution

Exposure of target tissues is, to a great extent, closely related to chemical solubility and lipophilicity. Water solubility tends to decrease with increasing degrees of chlorination, as does the extent and rate of biotransformation and elimination (Phillips, 1986). Thus, highly chlorinated congeners tend to be more persistent and have greater potential for bioaccumulation (Takagi *et al.*, 1986; Morrissey and Schwetz, 1989).

Once absorbed, PCBs are rapidly transported to all tissues by blood. The initial distribution of PCBs to tissues is determined by biophysical factors such as tissue volume, lipid content, tissue/blood partition ratios, binding to proteins, and perfusion rate (Maliwal and Guthrie, 1982; Matthews and Dedrick, 1984; Safe, 1989). Liver and muscle are the primary early depots for several reasons. The liver is highly perfused and has an affinity for these compounds; while muscle has the largest volume of all body tissues (Matthews and Anderson, 1975; Matthews and Dedrick, 1984). However, since PCBs are highly lipid-soluble compounds, they have a high affinity for lipid-rich tissues,

particularly adipose tissue. Simultaneously with initial distribution to the liver and muscles, redistribution to adipose tissue also occurs. These compounds are eventually concentrated in adipose tissue and skin with a dynamic equilibrium developing between all body tissues to maintain this concentration (Burse *et al.*, 1974; Matthews and Anderson, 1975; Lutz *et al.*, 1977). At equilibrium, a change in the PCB concentration of any tissue will result in a corresponding change in all tissues.

Metabolism

Metabolism and excretion are assumed to be important modes of detoxification of PCBs. Biotransformation of PCBs to hydroxylated metabolites by the microsomal cytochrome P-450 system is the critical event that determines the biological half-lives of these widespread environmental contaminants. The factors that determine the rate of biotransformation of PCBs are: the number of chlorines on the biphenyl nucleus, the position of these chlorines, and the animal species, age, and sex (Sipes and Schnellmann, 1987).

When gender and age were compared, enzyme induction was more pronounced in male rats than in females and increased with age (Chen and DuBois, 1973; McConnell, 1985). PCBs are generally metabolized to hydroxylated products either by direct oxygen insertion or via arene oxide intermediates into phenolic metabolites (Safe, 1984; Yoshimura *et al.*, 1987; Safe, 1989; Safe 1994). Two adjacent unsubstituted carbon atoms are important to the metabolism of PCBs because their presence facilitates the formation of arene oxides (Matthews and Tuey, 1980). Dechlorination can occur during further metabolism of the arene oxides (Hansen, 1987). The reactive arene-oxides

formed during biotransformation can bind covalently to tissue macromolecules or conjugate with glutathione. Therefore, the more readily metabolized congeners may pose a greater threat of toxicity than those that are more persistent, but slowly metabolized (Safe, 1984; Lutz and Dedrick, 1987).

Elimination and Excretion

In this review, excretion refers to removal of PCBs and their metabolites via feces and urine. Elimination, on the other hand, is the process through which PCBs are removed through gestation, parturition, and lactation. PCBs are passed from the mother to the fetus and carried in the offspring (Matthews and Dedrick, 1984).

PCBs are excreted to an appreciable extent only after they are metabolized and conjugated to form polar molecules. Derivatives of glutathione conjugates are major excretion products of PCBs as are glucuronides of the hydroxylated products (Matthews and Dedrick, 1984; Sipes and Schnellmann, 1987). They can also be metabolized by conjugation with cysteine and methionine, and then excreted in bile. Intestinal flora metabolize PCBs to stable methyl sulfides and methyl sulphones, which are mostly reabsorbed (Hansen, 1987). Methyl sulfide and methyl sulphone metabolites of PCBs are emerging as important contributors to food chain toxicities (Brandt *et al.*, 1982; Darnerud *et al.*, 1986; Hansen, 1987).

The majority of elimination is carried out through substances with high volume and lipid content, e.g. milk. Elimination by these routes is of great concern due to the possible threat to offspring (Matthews and Dedrick, 1984). Lower concentrations of PCBs are detected in umbilicus tissue, umbilicus blood and amniotic fluid than in

maternal blood. This is evidence of blood-placental barriers (Safe, 1989). In addition, the fetus is relatively lean compared with maternal tissues. Therefore, PCBs are not concentrated in fetal tissues.

The partition of PCBs is associated with physiological status. The creation of a new lipid depot with the onset of lactation results in passive movement of PCBs from blood to milk and a corresponding movement of PCBs from all tissues to blood in order to maintain their respective tissue/blood ratios (Matthews and Dedrick, 1984). Due to the placental barriers to PCBs and lipophilic properties of PCBs, transplacental exposure to PCBs is considerably less than exposure of the offspring through lactation (Takagi *et al.*, 1976; Bleavins *et al.*, 1984; Takagi *et al.*, 1986). Radioactivity was measured in mice treated with one dose of ^{14}C-6-CB (50 mg/kg bw) two weeks before mating (Gallenberg and Vodicnik, 1987). Almost all of the maternal body burden on day 19 of gestation was transferred to the offspring after 20 days of lactation (Gallenberg and Vodicnik, 1987). In addition, pregnant rats were treated orally with 1.33 mg ^{14}C-Kanechlor-400/kg bw once a week from day 8 of gestation to weaning. The concentrations of PCBs in the suckling rats increased gradually for 10 days after birth and then decreased gradually (Takagi *et al.*, 1976). The tissues measured included fat, muscle, bone, adrenal gland, blood, spleen, kidney, heart, and brain. The concentrations of PCBs in these tissues of suckling rats from day 1 through day 45 postpartum were higher than those in nursing dams sacrificed on day 23 of lactation.

When young adult rats were injected intravenously with a single dose of ^{14}C-TCB at 0.6 mg/kg bw (Abdel-Hamid *et al.*, 1981), cumulative excretion of radioactivity in

feces and urine reached 50% of the dose 1.5 days after administration (Abdel-Hamid *et al.*, 1981). Tissues samples were analyzed from 1 hour to 7 days after administration. TCB metabolites persisted in the blood over the 7-day period because concentrations were higher in this tissue than in liver, muscle, skin, and adipose tissue. Most of the radioactivity in rat tissues was parent TCB. Similar results were observed by Mizutani *et al.* (1977). The half-life of TCB in the carcass was 0.9 days when mature mice were fed 10 ppm TCB *ad libitum* for 20 days, and then given control feed. The TCB residue in the carcasses was analyzed daily for 4 days after TCB exposure.

Human Exposures and Clinical Investigations

Widespread environmental PCB contamination was first brought to the attention of the scientific community in the 1960s when PCBs were detected in fish, fish-spawn, eagles, and humans in Sweden (Anon, 1966). In 1968, accidental PCB contamination of rice oil occurred in Japan resulting in the poisoning of over 1000 individuals, which has been referred to as Yosho incident (Kashimoto and Miyata, 1986). Chloracne is a refractory follicular dermatitis characteristic of PCB poisoning in humans. This was observed in some Yosho patients 3 years after exposure to PCB-contaminated rice oil (Fishbein, 1974). A similar poisoning outbreak (Yu-Cheng) occurred in Taiwan in 1979 (Chen *et al.*, 1981). Yu-Cheng syndrome was characterized by acne-form skin eruptions, dark brown pigmentation of skin and nails, and hypersecretion of the Meibomian glands. Children born between 1978 and 1985 to Yu-Cheng mothers who were exposed to PCB-contaminated rice oil in 1978 and 1979 were investigated. The heights of Yu-Cheng children were 3.1 cm smaller than those in the control group. These children had less

total lean mass and soft tissue mass as compared to the matched controls. In addition, those musculoskeletal changes were only seen in the first born Yu-Cheng children after maternal ingestion of the contaminated rice oil, but not in subsequent siblings (Guo *et al.*, 1994).

Due to concern about the impact of industrial environmental contamination on nursing infants, PCBs concentration in human milk from the general population in industrialized regions have been compared with concentration in non-industrialized regions. In industrialized countries, PCB concentrations in human milk were 120-760 ppb, while those in the non-industrialized countries were 15-58 ppb (Schecter *et al.*, 1994). Similar results in New Zealand were reported by Bates *et al.* (1994) who compared PCB concentrations in human milk from urban and rural areas. Age was also associated with increasing concentrations of PCBs in milk (Bates *et al.*, 1994). The net absorption of PCBs in nursing infants was measured over 12 days. Over 95% of the PCBs in the milk was absorbed through the digestive tract (McLachlan, 1993). Some PCB congeners were also detected in both follicular and sperm fluids (Schlebusch *et al.*, 1989).

All of the PCB congeners have been assigned a toxic equivalent factor (TEF) value, which is the fractional toxicity of the congener relative to a standard toxin, TCDD. Toxic equivalents (TEq) are the sum of the concentrations of PCB congeners multiplied by their respective TEFs. Coplanar and mono-*ortho*-substituted congeners are the major sources of TEq in the blood of U.S. adults (Schecter *et al.*, 1994). Although coplanar PCBs are more toxic than other congeners, mono-*ortho*-substituted congeners

contribute more TEq than coplanar congeners (Schecter *et al.*, 1994). The authors suggested that in the general population of the United States, PCBs contribute more of the TEq in human tissue than do dioxins and furans. This is probably the case in other industrialized countries as well. There is increasing empirical evidence that human exposure to low levels of PCBs *in utero* and through nursing may result in subtle behavioral and developmental deficits (Jacobson *et al.*, 1983). Occupational neurobehavioral dysfunction was observed in firemen exposed to PCBs in transformer fires (Kilburn *et al.*, 1989).

General PCB Toxicity in Laboratory Animals

The adverse effects of PCB exposure in laboratory animals have been widely reviewed (Fishbein, 1974; Hansen, 1987; Parkinson and Safe, 1987). They are species, age, and sex dependent.

Wasting Syndrome

Wasting syndrome refers to progressive weight loss which is not totally related to decreased feed consumption. It appears to be due primarily to decreased feed efficiency, not consumption (Garthoff *et al.*, 1977). At lethal doses, the primary clinical sign in most species is a progressive loss of body weight followed by weakness, debilitation, and finally death. A steady loss of body weight was observed within the first 2 weeks of treatment when marmoset monkeys were orally dosed with 3 mg TCB/kg bw twice a week. Over a period of 18 weeks, body weight of the animals decreased by 34% (van den Verg *et al.*, 1988).

Dermal Toxicity

Nonhuman primates exhibit dermal lesions after exposure to low levels of PCBs. Alopecia, subcutaneous edema, and acne were observed in rhesus monkeys exposed to 2.5 ppm Aroclor 1248 for 6 months (Barsotti *et al.*, 1976). Atrophy and cystic dilation were observed in marmoset monkeys orally treated with 1 mg TCB/kg bw or 3 mg TCB/kg bw twice/week for 18-23 weeks (van den Berg *et al.*, 1988). Hyperplasia and hyperkeratosis of the epidermal and follicular epithelium were observed in rabbits after 38 days of dermal exposure to Phenoclor DP6, Clophen A 60 or Aroclor 1260 with a total of 27 applications of 118 mg/day (5 applications/week) (Vos and Beems, 1971).

Immunotoxicity

Thymic atrophy is a consistent result of TCDD and PCB exposure in all animals so far examined (Vos and Beems, 1973; Faith and Moore, 1977; Clarke *et al.*, 1984; van den Berg *et al.*, 1988; McConnell, 1989; Smialowicz *et al.*, 1989). The reduction in size is microscopically observed almost entirely as a loss of cortical lymphocytes (McConnell *et al.*, 1978; McConnell and Moore, 1979; van den Berg *et al.*, 1988). In mice receiving a single oral dose of 340 μg TCDD/kg bw, the thymus weight after 30 days was only 0.1 of the thymus weight of control animals (McConnell *et al.*, 1978). This decrease in thymus weight is often accompanied by necrotic debris in the medulla (McConnell, 1985). Rats were treated with 5 μg TCDD/kg bw either on days 0, 7, and 14 postnatally or on day 18 of gestation and days 0, 7, and 14 postnatally. Thymus/body weight ratios were found to be suppressed up to 39 days of age in those treated only postnatally. In those exposed to TCDD during gestation and postnatally, the thymic

effect was observed up to 145 days of age (Faith and Moore, 1977). Increased mortality was observed in young mallards co-exposed to PCBs and duck hepatitis virus (Friend and Trainer, 1970). Mortality increased markedly over the control value when ducklings were exposed to duck hepatitis virus after 10 days of 25, 50, or 100 ppm Aroclor 1254 treatment.

Hepatotoxicity

Liver enlargement was observed in PCB-exposed rats (Curley *et al.*, 1973; Garthoff *et al.*, 1977; Clarke *et al.*, 1984), mice (Sanders *et al.*, 1974), rabbits (Villeneuve and Grant, 1971), and mink (Aulerich *et al.*, 1985). Total liver lipid content, including cholesterol, phospholipid, and neutral lipid, increased in rats treated with 50 ppm or 500 ppm Aroclor 1254 for 3 weeks (Garthoff *et al.*, 1977). Total protein also increased with the 500 ppm treatment, while the relative total protein (mg/g wet weight) decreased with the same treatment. The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) is necessary for biosynthetic reactions, e.g. cholesterol, lipid, and some amino acid synthesis (Voet and Voet, 1990). The administration of 200 ppm Aroclor 1254 to rats for 3 and 14 days increased the activity of the major NADPH-generating enzymes: malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The activity of these enzymes in the liver increased approximately 2-fold above control activity (Hitomi *et al.* , 1993). PCB administration also causes a marked increase in serum cholesterol through the stimulation of hepatic cholesterogenesis (Kato and Yoshida, 1980). The rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase,

increased in rats fed 1000 ppm Aroclor 1248. Light microscopic examination of the livers of rats fed Aroclors 1242 and 1016 for 6 months showed enlarged hepatocytes with vacuolated cytoplasm and inclusions in the cytoplasm (Burse *et al.*, 1974). Bile-duct proliferation surrounded by fibrosis was also observed in 21-day-old weanling rats from dams given 50 mg Aroclor 1254/kg bw/day orally on days 7-15 of gestation (Curley *et al.*, 1973). Administration of 118 mg of Phenoclor DP6, Clophen A 60, or Aroclor 1260 (5 times per week, for 38 days) caused centrolobular degeneration, centrolobular liver cell atrophy, focal necrosis, and cytoplasmic hyalin degeneration in rabbits (Vos and Beems, 1971).

The cytochrome P-450 system is a group of membrane-bound enzymes located in the endoplasmic reticulum. Three coplanar congeners, TCB, P_5CB and HCB, bind to the A*h*-receptor, resulting in induction of cytochrome P-448-dependent hepatic microsomal monooxygenases (MC type). This binding elicits TCDD-like toxic effects (Lucier and McDaniel, 1979; Poland and Knutson, 1982; Nebert, 1989). 2,2',4,4'-Tetrachlorobiphenyl (4-CB) and 6-CB, not ligands of the A*h*-receptor, induce cytochrome P-450-dependent hepatic microsomal monooxygenases (PB type). This group of congeners has generally been proven to be less toxic than the coplanar PCB congeners (Lucier and McDaniel, 1979; Poland and Knutson, 1982; Parkinson *et al.*, 1983; Neal, 1985). Most congeners tested as well as Aroclor 1254 which is a commercial mixture, appear to be a combination of the MC and PB types: in that they induce both cytochromes P-448 and P-450 (Parkinson and Safe, 1981; Parkinson *et al.*, 1983).

Porphyria is a hepatic disorder distinguished by altered porphyrin metabolism

(Safe, 1984). The porphyrin biosynthesis involves several enzyme-catalyzed steps in which δ-aminolevulinic acid is ultimately converted into heme, an integral component of several enzymes, including the cytochrome P-450-dependent monooxygenases (Voet and Voet, 1990). The mechanisms leading to hepatic porphyria appear to be induction of δ-aminolevulinic acid synthetase (δ-ALAS)(Safe, 1984) and inhibition of uroporphyrinogen decarboxylase (Neal, 1985). Uroporphyrinogen decarboxylase is responsible for the stepwise decarboxylation of uroporphyrinogen to coproporphyrinogen (Voet and Voet, 1990). Together, induction of δ-ALAS and inhibition of uroporphyrinogen decarboxylase lead to increased hepatic and urinary porphyrins characterized by increased carboxyl groups (Safe, 1984; Neal, 1985).

Neurotoxicity

Prenatal exposure to TCB can also influence neurobehavioral functioning of mice during adulthood (Tilson *et al.*, 1979). Catecholamine levels have been shown to be altered in various brain regions of mink, doves, and rats exposed to Aroclor 1254 as well as to some individual congeners (Hansen, 1987). Decreased striatal dopamine concentration and receptor binding sites in the caudate nucleus were observed in mice at 1 year of age when those mice were treated with TCB *in utero* (Agrawal *et al.*, 1981). Impaired development of cliff avoidance reflexive behavior, swimming ability, and open field activity were found in offspring of PCB-exposed female rats when compared controls (Pantaleoni *et al.*, 1988).

Feed containing PCBs was fed to adult female mink for 1 month prior to breeding and through parturition (Aulerich *et al.*, 1985). Elevated hypothalamus concentrations

of both norepinephrine and dopamine were observed in the offspring, which could affect neuroendocrine control of reproduction.

Mutagenic and Carcinogenic Effects

PCBs are generally considered to be promoters rather than initiators (Hansen, 1987). The differential carcinogenicity of PCB congeners is dependent on a number of factors including the sex, strain, and species of the test animals (Safe, 1984). Experimental data show that commercial PCBs cause liver damage which leads to preneoplastic changes and hepatocellular carcinomas. The results also indicate that these lesions are only observed after long-term exposures to relatively high doses of the chemicals.

Reproductive Toxicity

Reproductive dysfunction as a result of PCB intoxication was first reported in women exposed to PCBs in 1968 in Japan. Dysmenorrhea and irregularities of the menstrual cycle were noted (Hirayama, 1976). Reproductive toxicity of PCBs received extensive experimental examination when early kit mortality was observed in domestic mink that were exposed to PCBs (Ringer *et al.*, 1972). Later studies revealed a correlation between early mink kit mortality and feed containing Great Lakes fish. In the past 20 years, PCB reproductive toxicity has been investigated in a large number of studies on humans, laboratory animals, and wildlife. Postnatal weight gain, neurobehavioral changes, and skin hyperpigmentation are the most sensitive end points of reproductive toxicity. A lowest observable adverse effect level (LOAEL) of 0.25 mg Aroclor 1254/kg bw/day was identified for rodents based on postnatal weight gain and

behavioral changes (Overmann *et al.*, 1987; Golub *et al.*, 1991).

Other toxic effects of PCBs on reproduction are changes in gonad weights, steroid hormone concentrations, and gamete production and fertilizing ability. These adverse effects are reviewed in more detail below.

Effects on Gonad Weights

PCBs have been reported to affect the weights of female and male reproductive organs. Increases in testis weights occurred in the 10-12-week-old offspring of mice gavaged with 20 mg 6-CB/kg bw every 3 days from day 13 of gestation through day 24 postpartum. This increase was not observed in mice gavaged with 40 mg 6-CB/kg bw or 160 mg Clophen A 50/kg bw every 2 days for 5 weeks, beginning at 5 weeks of age (Johansson, 1987). Nor were changes in testis weights observed when adult rats were treated with 500 ppm Aroclor 1254 in feed for 3 weeks (Garthoff *et al.*, 1977) or when adult mice were treated with 200 or 1000 ppm Aroclor 1254 in feed for 2 weeks (Sanders *et al.*, 1977). A decrease in testicular weight was noted in cockerels treated with 500 ppm Aroclor 1254 in the feed for 9 weeks from day of hatching (Platonow and Funnell, 1971). Decreased seminal vesicle weights were observed in white-footed mice treated with 200 ppm Aroclor 1254 in the feed for 4 weeks (Merson and Kirkpatrick, 1975).

Based on the observed changes in testis weights, animals exposed to PCBs perinatally are more susceptible to toxic effects than those animals treated during and after puberty. This may be related to the hormonal imbalance of the prenatal period when organogenesis occurs, thus impairing the development of reproductive organs.

Gellert (1978) investigated the uterotrophic activity of Aroclors 1221, 1242, 1254,

and 1260. Significant uterine growth 24 hours after treatment in weanling rats was only observed with a single subcutaneous injection of 1 mg Aroclor 1221/kg bw. This was not observed when rats were treated with Aroclors 1242, 1254, and 1260. Increased uterus weights were also observed with a single intraperitoneal (i.p.) injection of 8 mg/rat of Aroclors 1221, 1232, or 1248. In agreement with Gellert (1978), the highly chlorinated Aroclors 1254 and 1260 did not affect uterine weights (Ecobichon and MacKenzie; 1974). The increase in uterine weights appears to be associated with increased uterine glycogen content (Bitman and Cecil, 1970). Glycogen content in the uterus, an indicator of estrogenic activity, was measured in immature rats 18 hours after a single subcutaneous injection of 8 mg of Aroclor/rat (approximately 200 mg/kg bw). Increased glycogen content in uterus was detected in Aroclors 1221, 1232, 1242, and 1248, but not in Aroclors 1254, 1260, 1262, and 1268 (Bitman and Cecil, 1970).

According to the previous studies, exposure to the lesser chlorinated PCB mixtures is correlated with estrogenic activity than exposure to the more highly chlorinated mixtures. The lesser chlorinated PCBs are more rapidly metabolized and eliminated than higher chlorinated PCBs (Burse *et al.*, 1974; Safe, 1984). Compounds containing a phenol ring appear to bind to estrogen receptors. The phenol ring mimics the estrogen A ring and apparently promotes this binding (Duax and Griffin, 1985). The PCB metabolites that result from the rapid metabolism of lesser chlorinated PCBs contain hydroxyl groups. These create a structure that promotes binding to estrogen receptors and, therefore, cause an increase in estrogenic activity.

All the rats in the above studies were treated with a single injection at the age of

23-26 days which resulted in increased uterine weights. However, when adult mice were treated daily with 10 ppm Aroclor 1254 for up to 9 months, decreased uterine weights in the 8- and 12-week-old offspring were observed (Linzey, 1988). Exposure period appeared to be the primary contributor to differences in uterine weights. As with PCB effects on testis weights, multiple exposures during gestation cause impairment in the development of reproductive organs of offspring.

Effects on Steroid Hormones

During the prenatal period, testosterone is essential for the differentiation of male internal genital ducts. Dihydrotestosterone (DHT), a metabolite of testosterone, is responsible for differentiation of male external genitalia. During the early postnatal period, testosterone is converted to estrogen in the brain, which is critical for differentiation of the brain into the male type (Harris *et al.*, 1964; Hadley, 1992; Peterson *et al.*, 1993). Exposure to foreign compounds may alter neonatal androgen imprinting by changing the availability of steroid hormones during the critical developmental period, or by direct action of the xenobiotic on the hypothalamus and pituitary of neonates. The mechanism for decreasing plasma testosterone may involve induction of detoxification enzymes which also metabolize steroids. Altered hepatic microsomal testosterone hydroxylase activities were observed in adult male and female rats treated intraperitoneally with a single dose of approximately 32 mg Aroclor 1254/kg bw at 4 days postpartum (Haake-McMillan and Safe, 1991). Decreased plasma testosterone was observed in the 5- and 10-week-old male offspring of rats gavaged with 3 mg TCB/kg bw daily from day 6 through 18 of gestation (Vincent *et al.*, 1992). There

were, however, no significant changes in the plasma concentration of testosterone in the 10 to 12-week-old mouse offspring. Dams were gavaged with 20 mg 6-CB/kg bw every 3 days from day 13 of gestation through day 24 postpartum. The same results were observed in mice gavaged with 40 mg 6-CB/kg bw or 160 mg Clophen A 50 every 2 days for 5 weeks, beginning from 5 weeks of age (Johansson, 1987). The treatments had no effect on the synthesis of testosterone in testicular interstitial cells *in vitro* (Johansson, 1987).

Decreased testosterone concentrations were observed in the offspring of mice exposed to TCB on days 6 to 18 of gestation, but not in offspring of mice exposed to 6-CB from day 13 of gestation to day 24 after birth. In addition to treatment regimens, the results of these two studies may be due to the nature of the congeners; TCB is a 3-methylcholanthrene (3MC) type inducer while 6-CB is a phenobarbital (PB) type inducer. Decreased testosterone concentrations were not detected in animals exposed to PCBs during puberty or after puberty. This is supported by unaffected hepatic microsomal testosterone hydroxylase activity in postpubertal rats treated with a single i.p. injection of 32 mg Aroclor 1254/kg bw (Haake-McMillan and Safe, 1991). Although sample sizes in the Johansson study are larger than those in the Vincent *et al.* study, standard deviations in the former are larger than those in the latter. The large standard deviations in the former study could explain the lack of effect of treatment on testosterone concentrations.

Progesterone is imperative for the maintenance of pregnancy. Changes in progesterone concentration have been documented in PCB-treated female rhesus monkeys

(*Macaca mulatta*) and mink (Allen *et al.*, 1979; Aulerich *et al.*, 1985). A decrease in peak serum progesterone concentrations during the menstrual cycle was observed in adult female rhesus monkeys treated with 2.5 ppm (approximately 100 μg/kg bw/day) and 5.0 ppm Aroclor 1248 for 4 months (Allen *et al.*, 1979). However, when a daily oral dose of 0, 5, 20, 40, or 80 μg Aroclor 1254/kg bw was delivered in gelatin capsules for 2 years, serum progesterone concentrations in PCB-treated female adult rhesus monkeys were the same as those in the control group (Truelove *et al.*, 1990). Although the lowest dose in the Allen *et al.* study is only marginally higher than the highest dose in the Truelove *et al.* study, this may reflect the dose where treatment affects progesterone concentrations. In addition, the different results may be due to the nature of the mixtures, exposure routes, and length of treatment. Progesterone reduction is associated with high incidences of abortion in Aroclor 1248-exposed rhesus monkeys (Barsotti *et al.*, 1976). In adult female mink fed 2.5 ppm Aroclor 1254 beginning 1 month prior to breeding through parturition, decreased plasma progesterone concentration was noted. However, treatment with 0.1 ppm HCB resulted in an increase in progesterone concentrations (Aulerich *et al.*, 1985).

Decreased progesterone concentrations are probably caused by increased hydroxylation of progesterone. Although both Aroclor 1254 and HCB caused increased hepatic microsomal monooxygenases activity, decreased progesterone concentrations were only observed in Aroclor 1254-treated mink. These results may be due to the nature of the PCB fed; HCB is a 3-methylcholanthrene (3MC) type inducer while Aroclor 1254 is a mixed type inducer. Aulerich *et al.* (1985) suggest that the adrenal glands are the

source of the elevated plasma progesterone. This is supported by the increase in adrenal weights of the HCB-treated mink. Symptoms of debilitation such as lethargy and weakness were observed in these mink. This is probably associated with the increase in adrenal weights.

Although a dose-dependent *in vitro* breakdown of estradiol was observed in the livers of American kestrels treated with Aroclor 1254 or Aroclor 1262 for 5 months (Lincer and Peakall, 1970), changes in plasma estradiol were not observed in mammals exposed to PCBs. When adult female mink were fed any of the following treatments 1 month prior to breeding through parturition, plasma 17β-estradiol was not affected: 0.5 ppm HCB, 5 ppm 6-CB, 5 ppm 2,2',3,3',6,6'-hexachlorobiphenyl, or 2.5 ppm Aroclor 1254 (Aulerich *et al.*, 1985). Nor were any changes observed in circulating estrogen concentrations in the offspring of female rats gavaged with 3 mg TCB/kg bw/day for 13 days, beginning on day 6 of gestation (Vincent *et al.*, 1992).

Effects on Gamete Production

A reduction in testicular spermatozoa concentrations (sperm/mg testis) was observed in adult mice treated with 50 or 200 ppm Aroclor 1254 in the feed for 2 weeks after maturation (Sanders *et al.*, 1977). The number of sperm per testis was also reduced in adult mice with a dose of 400 ppm Aroclor 1254 in the feed for 2 weeks, but not with a dose of 100 or 200 ppm (Sanders and Kirkpatrick, 1975).

Decreased sperm production is accompanied by morphological changes in the testis. Derangement of seminiferous lobules and hyperplasia of lobule walls have been reported in Atlantic cod treated with 5 or 10 ppm Aroclor 1254 in the feed for 5.5

months (Freeman *et al.*, 1982). However, 5 daily i.p. injections of Aroclor 1254 at 125-1000 mg/kg bw in male mice caused no sperm head abnormalities when examined 5 weeks later (Topham, 1980).

A decrease in the number of oocytes and follicles was observed in the ovaries of 4-week-old C57/BL female mice exposed *in utero* via a single maternal i.p. injection of 15 mg TCB/kg bw (Ronnback, 1991). In contrast, an increased number of oocytes and follicles has been observed in the 8-week-old female offspring of dams exposed to 9 or 15 mg TCB/kg bw weekly. Treatment began 2 weeks prior to breeding and continued through gestation and lactation. No change of the number was observed when offspring were at 4 weeks of age. (Ronnback and de Rooij, 1994).

Effects on Gamete Fertilizing Ability

To determine effects of early postnatal exposure to PCBs on gamete quality, rats received 8, 32, or 64 mg Aroclor 1254/kg bw on days 1, 3, 5, 7, and 9 of lactation (Sager *et al.*, 1983). At 130 days of age, male offspring were mated with non-exposed females. A decrease in the number of implanted eggs and live fetuses, and an increased embryo resorption rate were noted. The authors speculated that this was caused by the hypoandrogenic effect of PCBs. In follow-up studies, a decrease in the percentage of normal fertilized eggs and eggs at the 2-cell, 4-cell, and blastocyst stages were observed (Sager *et al.*, 1987, 1991). In another study, when lactating mice were injected with 50 mg Clophen A 60/kg bw weekly for 3 weeks, with the first injection on the day of parturition, no effect on fertility was observed when exposed male offspring were mated to non-exposed mature females (Kihlstrom *et al.*, 1975). Although the dose (50 mg

Clophen A 60/kg bw) in this study was significantly higher than the LOAEL (8 mg Aroclor 1254/kg bw) in the Sager *et al.* studies, no effect on fertility was observed. The similarity in chlorination of Aroclor 1254 (54%) and Clophen A 60 (60%) suggests a similar toxic potency, but the results of this study do not appear to support this. However, the effects observed by Sager *et al.* could represent the relatively high exposure frequency of the Aroclor 1254 when compared to the Clophen A 60 treatment, rather than a difference in toxic potency between the two mixtures. In addition to a high exposure frequency, the rats in the Sager *et al.* studies received 4 treatments during the first week postpartum. This is a critical period for sexual differentiation of the brain and for the initiation of spermatogenesis. In contrast, the Kihlstrom *et al.* animals received only 2 treatments during this period.

In female mice, 10 weeks of daily doses as low as 1.25 mg Clophen A 60/kg bw reduced frequencies of implanted embryos. Treatment began 9 weeks prior to breeding and continued for 8-10 days of gestation (Orberg and Kihlstrom, 1973). Similar results were also observed in mice treated with individual congeners. A daily dose of approximately 25.0 mg 2,4',5-trichlorobiphenyl or 6-CB/kg bw, administered from day 1 to day 7 of gestation, resulted in decreased frequencies of implanted embryos (Orberg, 1978a).

Teratogenic and Developmental Toxicity

Commercial PCB mixtures have low teratogenic potency in laboratory animals. PCBs are thought to exert their teratogenic effects via similar mechanisms as TCDD, a widely recognized teratogen (Golub *et al.*, 1991). Individual coplanar congeners are

structurally similar to TCDD and thus are the most teratogenic of all the congeners (d'Argy *et al.*, 1987; Morrissey and Schwetz, 1989; Peterson *et al.*, 1993). Most structure-related overt teratogenic effects of PCBs are consistent with an A*h* receptor-mediated mechanism. Nevertheless, one exception is 2,2',3,3',4,4'-hexachlorobiphenyl, which, although having a very weak binding affinity for the A*h* receptor, causes the same pattern of teratogenic effects in mice as does TCDD (Marks and Staples, 1980). Coplanar congeners, more potent teratogens than other congeners, are present in low amounts in frequently tested commercial mixtures. This may account for the teratogenic potency of mixtures. Up to 65% malformations were seen in mice when dams were gavaged with 8 and 16 mg HCB/kg bw on days 6 through 15 of gestation (Marks *et al.*, 1981). Cleft palate and hydronephrosis were the dominant malformations detected. Teratogenicity of TCB was also investigated in mice (Simmons *et al.*, 1984; d'Argy *et al.*, 1987). The ED50 for cleft palate in mice is a single i.p. injection of approximately 100 mg TCB/kg bw (d'Argy *et al.*, 1987).

Sex ratios (♂/♀) of neonates decreased when pregnant rats were treated with a single dose of 3 mg TCB/kg bw on day 6 to day 18 of gestation (exact day not indicated) (Simmons *et al.*, 1984). This change appeared to be correlated with the tendency of TCB metabolites to accumulate in fetal tissue, as demonstrated with the following study. Three days after pregnant rats were administered ^{14}C-labeled 4-chloro-, 4,4'-dichloro-, 3,3',4,4'-tetrachloro-, 2,2',3,3',6,6'-hexachloro-, or 2,2',4,4',5,5'-hexachlorobiphenyl on day 18 of gestation, fetal blood levels of TCB radioactivity were higher than those of the other congeners (Lucier *et al.*, 1978).

PCBs have caused developmental toxicity in many laboratory animals. The signs of toxicity are similar to those that have been observed in humans (Hemminki and Vineir, 1985). These signs include neonatal death, growth retardation, hyperkeratosis, atrophy and cystic dilation of skin, and neurobehavioral dysfunction. In addition to these indications of toxicity, structural malformations have been observed in laboratory animals (Morrissey and Schwetz, 1989; Peterson *et al.*, 1993). Impaired reproductive performance and neurobehavioral effects are the most frequently observed functional alterations. Although decreased postnatal growth has been observed in most cases, increased postnatal growth rates were observed in mice treated daily with 0.05 mg 2,4',5-trichlorobiphenyl or 6-CB pre- and postnatally (Orberg, 1978b).

Decreased litter sizes at weaning were observed when wild-caught and laboratory-raised mice were treated with 10 ppm Aroclor 1254. It was suggested that exposure to PCB-contaminated foods in the field would contribute to a potential decline in the wild population of wild white-footed mice by reducing the number of young mice entering the breeding population (Linzey, 1987). When adult female mink were fed 0.5 ppm HCB beginning 1 month prior to breeding through parturition, all offspring died within 60 days of birth (Aulerich *et al.*, 1985).

3,3',4,4'-Tetrachlorobiphenyl (TCB) Toxicity

The toxicity of TCB, P_5CB, and HCB are frequently discussed together because they are all approximate isostereomers of TCDD. Cytochrome P-450 induced by either of these congeners or TCDD produces similar spectral maximal absorbances as cytochrome P-450 induced by 3-methylcholanthrene (3MC). These three congeners are,

therefore, known as MC-type congeners. The mechanism of toxicity of these coplanar congeners and TCDD is via A*h* receptors in the cytosol. After TCDD or coplanar congeners bind to the A*h* receptor, a receptor-ligand complex translocates into the nucleus. Following the interactions between this complex and genomic recognition sites, transcription and translation of the specific genes that encode for cytochrome P-448 activity are initiated (Sipes and Gandolfi, 1991). Since there is a high correlation between A*h* receptor binding affinity and toxicity, a toxic equivalency factor (TEF) approach has been adopted by regulatory agencies for the risk assessment of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and relevant compounds (Safe, 1994). All individual PCB congeners have been assigned a TEF value, which is the fractional toxicity of the congener relative to TCDD. Based on the mean of TEFs of over 10 responses elicited by each congener, the range of TEFs among those mono-*ortho* coplanar congeners is between 0.001 and 0.00005. The TEFs for TCB, P_5CB, HCB are 0.01, 0.1, and 0.05, respectively (Safe, 1994).

These 3 coplanar congeners are generally deemed highly toxic, with TCB being the least toxic of the three. After a single i.p. injection, ED50s of P_5CB, HCB, and TCB for body weight loss were 3.25, 15.1, and 730 μmol/kg bw, respectively (Leece *et al.*, 1985). This pattern was reflected by the induction of benzo[α]pyrene hydroxylase (BP hydroxylase), a MC-type hepatic microsomal enzyme. The increasing order of induction potencies of BP hydroxylase was TCB $<<$ HCB $\leq$ P_5CB (Yoshimura *et al.*,1979). The low toxicity of TCB when compared to the other 2 coplanar congeners corresponds with its relatively rapid biotransformation *in vivo* (Abdel-Hamid *et al.*, 1981; Mizutani *et al.*

1977).

The 2 TCB metabolites in rat feces, 5-hydroxy-TCB and 4-hydroxy-3,3',4',5-tetrachlorobiphenyl were considered less toxic than TCB due to their inability to cause liver enlargement, thymus atrophy, or induction of BP hydroxylase (Yoshimura *et al.*, 1987). Similar results were observed in the comparison between the toxicity of P_5CB and its metabolite 4'-hydroxy-P_5CB (Koga *et al.*, 1990). Chicken embryo mortality was not affected by a dose of 2.0 mg hydroxylated metabolites of TCB/kg egg (Klasson Wehler *et al.*, 1990). In contrast, 75% mortality was reported for chicken embryos injected with 0.02 mg TCB/kg egg *in ovo*. These studies demonstrate the considerable toxicity of TCB when compared to the inability of TCB metabolites to elicit toxic effects.

HYPOTHESIS

Phase I

The first hypothesis is that the TCB tissue concentrations will be relatively higher in the low-dose group than the tissue concentrations in the high-dose group. This is derived from the fact that high concentrations of PCBs can induce hepatic microsomal monooxygenases to a greater extent than low concentrations.

The second hypothesis is that accumulation of TCB is higher in pregnant mice than in non-pregnant mice. This is based on the observation that the PCB-induced increase in hepatic microsomal monooxygenases was less in pregnant mice than in virgin animals.

Phase II

The hypothesis is that exposure to TCB will impair both reproductive performance and gamete integrity in mice. PCBs induce hepatic microsomal monooxygenases that can metabolize steroid hormones which are essential for prenatal and early postnatal imprinting and development of accessory sex organs. The imbalance of steroid hormones can elicit adverse effects on organogenesis and gametogenesis.

MATERIALS AND METHODS

Phase I

Animals

Twenty four C57BL/6J female mice at 15 weeks of age were used in this phase. Mice were housed in a containment room at University Laboratory Animal Resources, Michigan State University. Room temperature was maintained at 70-72°F with 40-60% humidity and 12 hour light/12 hour dark cycle.

Chemicals

UL-^{14}C-TCB (Lot # 026F9204; 50 μCi/50 μl toluene) was purchased from Sigma Chem. Co., St. Louis, MO. Specific activity of ^{14}C-TCB was 37.1 mCi/mmol and chemical purity exceeded 98%. TCB (Lots # 70075 and 10162; purity > 99.4%) was purchased from AccuStandard Inc., New Haven, CT. Liquid scintillation cocktail was purchased from Beckman Instruments, Inc., Fullerton, CA. Acetonitrile (HPLC grade) was purchased from EM Science, Gibbstown, NJ. Acetone was purchased from J. T. Baker Inc., Phillipsburg, NJ.

Feed Preparation

In this feeding trial, ^{14}C-TCB was used to measure placental transfer of TCB as well as distribution of TCB in tissues. Two types of feed were prepared: ^{14}C-TCB feed (^{14}C-TCB and TCB) and TCB feed.

^{14}C-TCB Feed

Feed contained 0, 3 ppm, or 30 ppm total TCB. Purchased ^{14}C-TCB (50 μCi/50 μl) was diluted by a factor of 20 with toluene to make a 50 μCi/1 ml stock solution. Acetone was used as a carrier to disperse ^{14}C-TCB and TCB in ground mouse chow 5015 (PMI Feeds, Inc., St. Louis, MO). After 9.52 μCi ^{14}C-TCB in 190.5 μl toluene and TCB were dissolved in 60 ml of acetone, 1 kg ground mouse chow was added and mixed. The specific radioactivity was 3.17 and 0.317 μCi/mg TCB in 3 ppm and 30 ppm ^{14}C-TCB feed, respectively. The feed was tumbled for 1 hour and air dried in a hood at room temperature for 24 hours, before being stored in glass jars in a 0-4°C refrigerator until use. The control feed consisted of ground mouse chow mixed with acetone and toluene and similarly tumbled and dried. Feed samples were analyzed by the laboratory of Dr. Thomas Voice, Department of Civil and Environmental Engineering, Michigan State University, at the onset of this study.

TCB Feed

Feed containing TCB only was prepared using the same procedure described above but without ^{14}C-TCB. The feed was prepared monthly.

Treatments

Before mating, 8 female C57BL/6J mice (F-0) at 15 weeks of age were randomly assigned to each treatment and provided with ^{14}C-TCB feed *ad libitum* for 2 weeks. Each female was then paired with one non-treated mature C57BL/6J male for 10 days between 7:30 PM and 7:30 AM, during which time feed was withheld to avoid exposing males to treatment feed. The presence of the vaginal plug was defined as day 1 of

gestation. F-0 females continued on the ^{14}C-TCB treatment until parturition and were then switched to a TCB diet for the next 21 days. This study was terminated 21 days postpartum when the offspring were weaned.

Sample Collection

On day 19 of gestation, liver, thymus, uterus, abdominal adipose tissue, placenta, and fetus samples were collected and weighed. These same tissues, except uterus, placenta, and fetus, were also collected on day 21 of lactation. One animal from each treatment group was used for sample collection. One non-pregnant mouse from each treatment was sacrificed concurrently with the animals on day 19 of gestation and day 21 of lactation. The liver, thymus, and abdominal adipose tissue of 3-week-old male offspring were collected, weighed and stored for subsequent analysis.

Sample Extraction and Analysis

Each sample was prepared for TCB and radioactivity analysis by disruption in 10 ml acetonitrile with a Dismembrator Sonic (sonicator model 550, Fisher Scientific, Pittsburgh, PA) at 20% power for 10 minutes. The sample was then centrifuged at 1500 RPM for 10 minutes. Ten ml liquid scintillation cocktail was added to 5 ml supernatant for the measurement of radioactivity with a liquid scintillation counter (Model 1500 Tri-Carb, Packard Instrument Company, Meriden, CT). The concentrations of TCB equivalents in the tissues and organs were calculated from the radioactivity measured in the tissues and organs and the specific activity in feed (Appendices I and II). The term TCB equivalents refers to TCB and its metabolites.

Phase II

Animals

Ninety C57BL/6J female mice at 13 weeks of age were used in this phase. Non-treated B6D2-F1 males were used as sperm donors for the *in vitro* fertilization assay. Mice were raised in the same facility as in Phase I.

Chemicals

TCB was purchased from AccuStandard Inc., New Haven, CT. Pregnant mare's serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), and all other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Feed Preparation

The method of feed preparation was the same as described in Phase I.

Treatments

Female C57BL/6J mice (F-0) were fed Purina lab chow containing 0, 3 ppm, and 30 ppm TCB for 2 weeks beginning at 13 weeks of age. Each female was then paired with one non-treated mature C57BL/6J male for 10 days, between 7:30 PM and 7:30 AM, during which time feed was withheld to avoid exposing males to treatment feed. The treatment for F-0 females continued through mating, gestation, and lactation. The offspring (F-1) were given the same feed as their dams received throughout the study. Females (F-1) were superovulated at 5, 6, and 7 weeks of age and egg fertilizing ability was assessed by *in vitro* fertilization (IVF) assay. At 7 weeks of age, 10 F-1 females in each treatment group were bred with non-treated mature B6D2-F1 males for 10 days. Ten treated F-1 males in each treatment group, aged 7 and 17 weeks, were used to breed

non-treated mature B6D2-F1 females for 10 days. After breeding, sperm from these males were collected and fertilizing ability was assessed by IVF assay. Determination of sperm concentration and sperm motion analysis were performed on sperm from 9-week-old F-1 males hourly for 4.5 hours after sperm collection with the CellSoft computer-assisted digital image analysis system (CRYO Resources Inc., New York, 1986).

Parameters Measured

Feed Intake Feed intake was recorded for F-0 females before mating, during gestation, and during lactation. The amount of feed added every day was recorded. Actual feed consumption was calculated every 3 days by subtracting the amount of feed left in the feed jars from the total feed provided over the 3-day period.

Body Weight Body weights of F-0 females were recorded at the initiation of the study (day 1), before breeding (day 14), on day 19 of gestation (day 33), and on day 21 of lactation (day 54). When the offspring were 4 days, 1 week, and 2 weeks of age, body weights were recorded by litters. The average weight of offspring in a litter was used for statistical analysis. After weaning, individual weights were recorded weekly.

Organ Weight Liver and thymus weights of F-0 females were recorded for animals on day 19 of gestation and on day 21 of lactation. Liver and thymus weights of F-1 females were recorded at 5, 6, and 7 weeks of age. Liver, thymus, and testis weights of F-1 males were determined at 3, 9, and 19 weeks of age.

Fecundity, Litter Size, Sex Ratio, and Survival Index Fecundity, litter sizes, sex ratios, and 4-day and 21-day survival indices were recorded for both F-0 and F-1

generations. Sex of pups was determined by anogenital distances at day 4 of age or at death if they died before day 4 of age. If pups died before sex could be determined, they were not included. A 4-day survival index is defined as 100 × [number of pups viable at day 4 of age]/[number of viable pups born]. A 21-day survival index is defined as 100 × [number of pups viable at day 21 of age]/[number of pups retained at day 4 of age] (Thomas, 1991).

In Vitro Fertilization Treated F-1 females were superovulated with 8 IU PMSG, and then 8 IU HCG 48-50 hours later. Thirteen hours after HCG injection, the females were killed by cervical dislocation. Oviducts and distal portions of the uteri were excised. The eggs were collected by gentle teasing from the oviducts and incubated 5-15 minutes prior to insemination. Epididymal sperm from non-treated B6D2-F1 males were collected and incubated for 1.5 hours before insemination. Eggs in each petri dish, collected from one female, were inseminated with 50 μl sperm suspension (approximately 6×10^5 sperm) and incubated for 24 hours. Incubation occurred in modified Tyrodes medium at 37°C and 5% CO_2 after collection and insemination. Twenty-four hours after insemination, 50 μl of a 35 μM solution of bisBenzimide (Hoechst 33258) were added to the petri dish. It was incubated for another 30 minutes, and then observed under a Nikon Optiphot microscope equipped with a 100w mercury bulb, 365/10 nm excitation filter, 400 nm dichroic mirror, and 400 nm barrier filter. Eggs at the 2-cell stage or eggs containing a second polar body and two pronuclei were considered fertilized. Degenerated eggs were considered non-fertilized.

The IVF assay was also used to evaluate sperm fertilizing ability of treated males.

The method was the same as described above. Epididymal sperm from treated males at 9 and 19 weeks of age were collected and used to inseminate eggs from non-treated B6D2-F1 mice.

Culture Medium Modified Tyrodes media contained 99.23 mM sodium chloride, 2.68 mM potassium chloride, 1.80 mM calcium chloride, 0.36 mM sodium phosphate monobasic anhydrous, 0.49 mM magnesium chloride hexahydrate, 25.0 mM sodium bicarbonate, 52.0 mM sodium lactate, 0.25 mM sodium pyruvate, 5.56 mM D-glucose, 100 IU/ml sodium penicillin G., and 100 IU/ml streptomycin sulphate.

Sperm Concentration and Motion Analysis Twenty μl of the sperm suspension (approximately 2.4×10^5 sperm/ml) were placed on a CellSoft 20 μm chamber and analyzed with the CellSoft computer-assisted digital image analysis system. A minimum of 100 sperm cells were analyzed to obtain measurements of concentration, motility, velocity, linearity, mean amplitude of lateral head (ALH) displacement, and beat/cross frequency. Motility is the percentage of sperm that travel more than 20 μm/sec. Velocity is the average distance (μm) traveled by motile sperm in one second. Linearity is a measure of whether or not the sperm move in a straight line. It is calculated by dividing the length of the straight line distance by the actual track distance. This fraction is then multipled by 10. Linearity, therefore, ranges from 0 to 10. The straighter the actual cell track, the higher the linearity value such that a value of 10 indicates a perfectly straight line and 0 indicates a circular track. ALH displacement is a measure of the displacement of the sperm head from a computer-calculated curval mean of its track. The maximum ALH displacement is measured from the curval mean for every

cycle of the cell's track, then multipled by 2. For the individual cell data, the mean ALH displacement is the mean of the maximum ALH displacement for the cycles of the track. For the summary data, the mean ALH displacement is an average of the mean ALH displacement for each cell. High ALH displacement appears to be linked with the process of capacitation. The beat/cross frequency (Hz) is the number of beats (or crosses) per second. Every time the sperm cell crosses the computer-calculated curval mean, the computer counts that crossing as one beat.

Statistical Analysis

Fecundity, survival indices, fertilization rate, degeneration rate, and sperm motility were evaluated with the Chi square test. Body weight, organ weight, feed intake, and ALH displacement were initially tested with One Way Analysis of Variance (ANOVA). Log transformation was performed for the discrete quantitative parameters and ratios (i.e., litter size, number of eggs ovulated, sperm concentration, beat/cross frequency, sperm linearity, sex ratios, and relative organ weights) before ANOVA. Relative organ weights refer to organ weights as percentages of body weights. Those data passing homogeneity of variance and normality tests were analyzed with the Student-Newman-Keuls multiple pairwise comparison. The Kruskal-Wallis Test was performed to evaluate the non-Gaussian data. These analyses were performed with Sigmastat® (Kuo *et al.*, 1992).

RESULTS

Phase I

Results of feed analysis are shown in Table 1. TCB concentrations were approximately 80% of expected concentrations in Phase I and over 90% in Phase II.

After 5 weeks of exposure, TCB equivalents in the liver, adipose tissue, uterus, and fetus from the 30 ppm group were 3-6 fold higher than the levels in the 3 ppm group, except for the uterus in the non-pregnant groups (Table 2). Concentrations of TCB equivalents were similar in the uterus, placenta, and fetus in the 30 ppm group (Table 2). No placenta data was available in the 3 ppm group.

After 8 weeks of ^{14}C-TCB treatment, TCB equivalents were still accumulating in the liver and adipose tissue of non-pregnant mice, in both the low and high dose groups (Table 3). TCB equivalents in the thymus of lactating mice were higher than those in the non-pregnant mice, regardless of dose (Table 3). TCB equivalents in the thymus of 30 ppm- treated lactating mice were over 40 times higher than equivalents in the 3 ppm-treated lactating mice (Table 3).

In the 3 ppm-treated group, approximately 15% of the concentrations of TCB equivalents that had accumulated in the liver and adipose tissue before parturition were detected after termination of lactation (Tables 2 and 3). However, in the 30 ppm-treated group, approximately 56% and 4%, respectively, of the concentrations of TCB

equivalents that had accumulated in the adipose tissue and liver before parturition were observed after termination of lactation (Tables 2 and 3).

After weaning, radioactivity was only detected in the liver and adipose tissue, not in the thymus of the offspring (Table 4). Similar concentrations of TCB equivalents were detected in the adipose tissue and liver of the 3 ppm-treated offspring. However, in the 30 ppm group, concentrations of TCB equivalents in the adipose tissue were 14.6 times higher than concentrations in the liver (Table 4). Although the pups were nursed by the same dam in the 30 ppm group, large variations in the concentrations of TCB equivalents were observed (Table 4).

Table 1. TCB concentrations in treatment feed.

	Treatments		
	Control	3 ppm	30 ppm
		Concentration (ppm)	
Phase I[a]	0 (n=2)	2.36 ± 0.27 (n=3)	23.49 ± 1.18 (n=3)
Phase II[b]	0 (n=3)	3.16 ± 0.24 (n=3)	27.59 ± 0.70 (n=3)

a The TCB concentrations in feed were calculated from the specific activity in feed and the radioactivity measured in the feed with a liquid scintillation counter (Appendices I and II).

b TCB concentrations in Phase II were analyzed by gas chromatography.

Table 2. Concentrations of TCB equivalents in tissues and organs of non-pregnant and pregnant mice.

Organ/ Tissue	Concentration[a]	Treatments			
		Non-pregnant[b]		Pregnant[b]	
		3 ppm	30 ppm	3 ppm	30 ppm[c]
Adipose Tissue	ppm	0.88	4.86	0.99	3.26
Liver	ppm	0.94	3.23	0.92	5.71
Thymus	ng TCB/ Thymus	4.4	25.2	2.5	15.8
Uterus	ppm	0.29	0.21	0.12	0.54
Placenta	ppm	NA[d]	NA	-[e]	0.61
Fetus	ppm	NA	NA	0.14 ± 0.03 (n=8)	0.47 ± 0.13 (n=21)

a The term equivalents refers to TCB and its metabolites. The concentrations of TCB equivalents in organs and tissues were calculated from the specific activity in feed and the radioactivity measured in the organs and tissues with a liquid scintillation counter (Appendices I and II).

b Both non-pregnant and pregnant mice were treated with ^{14}C-TCB feed for 5 weeks.

c Values are the means of data from 2 pregnant mice in the 30 ppm group. Table values from all other groups reflect a sample size of one.

d Not applicable.

e Missing data.

Table 3. Concentrations of TCB equivalents in adipose tissue and organs of non-pregnant and lactating mice.

Organ/ Tissue	Concentration[a]	Treatments			
		Non-pregnant[b]		Lactating[b]	
		3 ppm	30 ppm	3 ppm	30 ppm
Adipose Tissue	ppm	1.26	8.71	0.14	1.83
Liver	ppm	2.29	5.98	0.15	0.24
Thymus	ng TCB/ Thymus	0	132.1	4.8	205.2

a The term equivalents refers to TCB and its metabolites. The concentrations of TCB equivalents in organs and tissues were calculated from the specific activity in feed and the radioactivity measured in the organs and tissues with a liquid scintillation counter (Appendices I and II).

b Non-pregnant mice were treated with ^{14}C-TCB feed for 8 weeks. Lactating mice were treated with ^{14}C-TCB feed for 5 weeks before parturition, and TCB feed for 3 weeks after parturition. Table values reflect a sample size of one.

Table 4. Concentrations of TCB equivalents in adipose tissue and organs of male offspring at 3 weeks of age.

Organ/ Tissue	Concentration[a]	Treatments	
		3 ppm (n=4)	30 ppm (n=5)
Adipose Tissue	ppm	0.15 ± 0.03	8.15 ± 7.84
Liver	ppm	0.10 ± 0.01	0.56 ± 0.14
Thymus	ng TCB/ Thymus	0	0

a The term equivalents refers to TCB and its metabolites. The concentrations of TCB equivalents in organs and tissues were calculated from the specific activity in feed and the radioactivity measured in the organs and tissues with a liquid scintillation counter (Appendices I and II). The calculated concentrations of TCB equivalents were transferred through the placenta and milk from the dams which were treated with ^{14}C-TCB feed for 5 weeks before parturition, and then TCB feed for 3 weeks after parturition.

Phase II

After the first two weeks of treatment, there were no significant differences in body weights in the F-0 mice, although mice in the 30 ppm group consumed more feed than those in the 3 ppm group (Table 5). Feed intake during gestation days 1 - 16 was also higher in the 30 ppm group than in the 3 ppm group, although there were still no significant differences in body weights between the 2 groups. On day 21 of lactation, however, the 3 ppm TCB-treated mice consumed more feed than the 30 ppm-treated mice, although they weighed less than the 30 ppm-treated mice (Table 5). On day 19 of gestation and on day 21 of lactation, relative liver weights of F-0 mice were higher in the 30 ppm treatment group than in the 3 ppm and the control groups (Table 6). These differences were not apparent in non-pregnant females. TCB treatments had no significant effect on thymus weights in F-0 females (Table 6).

Fecundity in F-0 females was 80%, 71%, and 47% in the control, 3 ppm, and 30 ppm groups, respectively (Table 7). The treatments had no effect on litter sizes and sex ratios. Four-day and 21-day survival indices showed a trend of dose-response relationships. Decreased survival was observed in the 30 ppm group, in which most of the mortality occurred before 4 days of age (Table 7). There were no significant differences in body weights of F-1 offspring (Table 8). At 5 and 6 weeks of age, F-1 females in the 30 ppm group showed an increase in liver weights, but not in relative liver weights (Table 9). A decrease in thymus weights was observed in the 30 ppm-treated F-1 females between 5 and 7 weeks of age (Table 9). However, relative thymus weights decreased in the 30 ppm-treated F-1 females only at 5 weeks of age.

Table 5. Effects of TCB on feed intake and body weights of F-0 female mice.

	Treatments		
	Control	3 ppm	30 ppm
Body Weight (g)[c] (13 Weeks of Age)	21.6 ± 0.8 (n=18)	21.7 ± 0.7 (n=24)	21.8 ± 1.0 (n=46)
Feed Intake (g/day)[d] (13-15 Weeks of Age)	4.7 ± 0.9[ab] (n=15)	4.4 ± 0.8[a] (n=15)	5.2 ± 0.6[b] (n=15)
Body Weight (g)[c] (15 Weeks of Age)	21.7 ± 0.9 (n=18)	21.6 ± 0.5 (n=24)	22.2 ± 1.1 (n=46)
Feed Intake (g/day)[e] During Gestation	4.8 ± 1.2 (n=7)	4.6 ± 1.4 (n=7)	5.4 ± 1.0 (n=4)
Body Weight (g) Day 19 of Gestation	36.9 ± 2.1 (n=3)	37.3 ± 1.2 (n=3)	35.6 ± 2.9 (n=3)
Feed Intake (g/day)[f] During Lactation	8.8 ± 2.3[a] (n=9)	10.3 ± 1.6[a] (n=8)	7.7 ± 2.3[b] (n=10)
Body Weight (g) Day 21 of Lactation	28.5 ± 1.6[ab] (n=3)	26.2 ± 0.1[a] (n=3)	29.3 ± 1.6[b] (n=3)

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c Females were treated with TCB 2 weeks beginning at 13 weeks of age. Each female was then paired with one non-treated mature male for 10 days.

d Values represent mean feed intake ± SD during the two weeks before mating.

e Values represent mean feed intake ± SD from day 1 to 16 of gestation.

f Values represent mean feed intake ± SD from day 1 to 21 of lactation.

Table 6. Effects of TCB on organ weights and relative organ weights of F-0 females.

Weight[c]	Treatments		
	Control (n=3)	3 ppm (n=3)	30 ppm (n=3)
Non-pregnant (18 Weeks of Age)			
LW (g)	1.33 ± 0.36	1.08 ± 0.04	1.47 ± 0.55
LW/BW[d] × 100	5.73 ± 1.24	5.03 ± 0.11	6.62 ± 2.65
TMW (g)	24.4 ± 8.4	32.4 ± 5.3	36.8 ± 9.8
TMW/BW[d] × 100	0.11 ± 0.04	0.15 ± 0.03	0.16 ± 0.04
Day 19 of Gestation			
LW (g)	1.69 ± 0.07	1.81 ± 0.12	1.82 ± 0.13
LW/BW[e] × 100	4.59 ± 0.09[a]	4.84 ± 0.23[a]	4.95 ± 0.07[b]
TMW (mg)	19.0 ± 9.2	25.6 ± 7.0	18.0 ± 1.4
TMW/BW[e] × 100	0.06 ± 0.03	0.07 ± 0.02	0.05 ± 0.003
Day 21 of Lactation			
LW (g)	2.06 ± 0.16[ab]	1.99 ± 0.11[a]	2.37 ± 0.20[b]
LW/BW[e] × 100	7.22 ± 0.34[a]	7.60 ± 0.42[a]	8.10 ± 0.32[b]
TMW (mg)	34.4 ± 2.3	29.0 ± 5.9	31.7 ± 9.6
TMW/BW[e] × 100	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.03

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c BW, LW, and TMW represent body, liver, and thymus weights, respectively. Values represent mean organ weights or organ weights as percentages of body weights ± SD.

d Body weights were 23.1 ± 1.2, 21.5 ± 0.5, 22.2 ± 0.5 g in control, 3 ppm, 30 ppm group, respectively.

e Body weights were based on the data in Table 5.

Table 7. Effects of TCB on reproductive performance of F-0 female mice.

	Treatments		
	Control	3 ppm	30 ppm
Fecundity (%)[c]	80[a]	71[a]	47[b]
Litter Size	8.3 ± 2.0 (n=12)	8.3 ± 1.3 (n=16)	8.2 ± 1.2 (n=20)
Sex Ratio (♀/♂)	1.11 ± 0.51 (n=12)	1.09 ± 0.72 (n=16)	1.05 ± 1.00 (n=20)
4-Day Survival Index[d]	47.4 ± 34.7[a] (n=12)	41.6 ± 39.1[a] (n=16)	30.9 ± 30.9[b] (n=20)
21-Day Survival Index[e]	96.3 ± 11.1[a] (n=9)	88.0 ± 31.6[a] (n=10)	75.8 ± 37.7[b] (n=13)

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c Values are expressed as percentages of females mated which became pregnant.

d Four-day survival index = 100 × [number of pups viable at day 4 of age]/[number of viable pups born].

e Twenty-one-day survival index = 100 × [number of pups viable at day 21 of age]/[number of pups retained at day 4 of age]. The litters in which all pups died before day 4 of age were not included.

Table 8. Effects of TCB on body weights of F-1 mice.

Age[a]	Treatments		
	Control	3 ppm	30 ppm
		BW (g)	
Day 4	1.8 ± 0.3 (n=9)	1.8 ± 0.4 (n=10)	1.7 ± 0.3 (n=13)
Week 1	3.3 ± 0.7 (n=9)	3.6 ± 0.4 (n=9)	3.1 ± 0.6 (n=12)
Week 2	6.9 ± 1.4 (n=9)	7.3 ± 0.6 (n=9)	6.5 ± 1.5 (n=12)
Week 3	7.9 ± 1.2 (n=45)	8.4 ± 0.7 (n=51)	7.6 ± 2.4 (n=37)
Week 4	14.5 ± 1.3 (n=42)	14.7 ± 2.8 (n=51)	14.9 ± 3.5 (n=32)
Week 5	19.3 ± 2.4 (n=32)	19.0 ± 3.2 (n=47)	19.0 ± 3.6 (n=29)
week 6	20.0 ± 2.5 (n=41)	20.1 ± 3.2 (n=49)	20.6 ± 3.1 (n=27)
Week 7	21.7 ± 3.1 (n=33)	21.2 ± 3.5 (n=45)	21.3 ± 3.3 (n=21)

a Body weights on day 4, week 1, and week 2 represent the means ± SD of litters. Values for week 3 through week 7 are means ± SD of individual weights.

Table 9. Effects of TCB on body weights, organ weights, and relative organ weights of F-1 females between 5 and 7 weeks of age.

Age	Treatments		
	Control	3 ppm	30 ppm
	BW (g)		
Week 5	17.8 ± 0.5 (n=7)	18.3 ± 1.5 (n=9)	18.3 ± 1.8 (n=8)
Week 6	18.1 ± 0.7 (n=6)	18.9 ± 0.2 (n=3)	18.4 ± 0.1 (n=2)
Week 7	19.6 ± 0.7 (n=7)	19.8 ± 1.0 (n=10)	18.9 ± 1.2 (n=4)
	Relative Liver Weight (LW/BW × 100)[c] (LW (g))[d]		
Week 5	6.26 ± 0.53 (1.11 ± 0.08[a])	6.09 ± 0.47 (1.11 ± 0.14[a])	7.05 ± 0.42 (1.30 ± 0.17[b])
Week 6	6.00 ± 0.23 (1.09 ± 0.07[a])	5.84 ± 0.33 (1.10 ± 0.07[a])	7.04 ± 0.37 (1.30 ± 0.08[b])
Week 7	5.75 ± 0.60 (1.13 ± 0.14)	6.06 ± 0.38 (1.20 ± 0.12)	6.35 ± 0.39 (1.26 ± 0.15)
	Relative Thymus Weight (TMW/BW × 100)[c] (TMW (mg))[d]		
Week 5	0.44 ± 0.88[ab] (77.7 ± 12.0[a])	0.46 ± 0.05[a] (81.0 ± 7.0[a])	0.36 ± 0.03[b] (60.8 ± 5.9[b])
Week 6	0.43 ± 0.08 (77.5 ± 13.7[a])	0.42 ± 0.09 (80.1 ± 16.9[ab])	0.30 ± 0.02 (55.1 ± 2.3[b])
Week 7	0.39 ± 0.08 (75.2 ± 11.7[a])	0.37 ± 0.08 (67.8 ± 9.8[ab])	0.30 ± 0.06 (55.8 ± 9.4[b])

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c BW, LW, and TMW represent body, liver, and thymus weights, respectively. Values represent mean of organ weights as percentages of body weights ± SD.

d The numbers in the parentheses under relative organ weights represent organ weights ± SD.

Decreases in fertilizing ability of F-1 eggs *in vitro* were observed in the 3 ppm and 30 ppm groups when compared to the control group (Table 10). Fertilization rates were 68.4%, 47.6%, and 45.6% for the control, 3 ppm, and 30 ppm groups, respectively. The percentage of degenerated eggs in the 3 ppm and 30 ppm groups was higher than that in the control group. The number of eggs superovulated by exogenous PMSG and HCG were the same among all groups (Table 10). F-1 females at 7 weeks of age showed similar fecundity among all groups when bred with non-treated males (Table 11). Nonetheless, all offspring from 3 ppm and 30 ppm-treated F-1 females died within 4 days of birth (Table 11).

Testes in treated F-1 males at 3 weeks of age were heavier in mice treated with 30 ppm TCB than for mice in the control group (Table 12). However, relative testis weights were higher in both 3 ppm and 30 ppm groups than in the control group. Liver weights, relative liver weights, thymus weights, and relative thymus weights were similar among all groups (Table 12). At 9 and 19 weeks of age, liver weights and relative liver weights were higher in the 30 ppm group than in the other 2 groups. However, thymus and testis weights were the same among all 3 groups (Tables 13 and 14). Treated F-1 males, at 7 and 17 weeks of age, were bred with non-treated females. Fecundity, litter sizes, sex ratios, and survival of the offspring were the same among all treatment groups (Tables 15 and 16). The 4-day survival index decreased by 20% in the 3 ppm and 30 ppm groups when treated males at 60 weeks of age were paired with non-treated females (Table 17).

Table 10. Fertilizing ability of eggs from F-1 females exposed to TCB *in utero* through postnatal development.

	Treatments		
	Control	3 ppm	30 ppm
No. Females Tested	15	16	12
No. Exp.	6	6	6
Eggs Ovulated Per Animal (No.)	40 ± 10	29 ± 21	34 ± 22
Fertilization *In Vitro* (%)	68.4 ± 15.1[a]	47.6 ± 25.9[b]	45.6 ± 18.7[b]
Degeneration Rate (%)	6.0 ± 4.8[a]	15.6 ± 20.2[b]	13.4 ± 7.9[b]

ab Different superscripts represent significant differences within the same row ($P < 0.05$)

Table 11. Effects of TCB on reproductive performance of F-1 females at 7 weeks of age.

	Treatments		
	Control	3 ppm	30 ppm
No. Females Mated	10	10	10
Fecundity (%)	50	40	70
Litter Size	7.0 ± 2.3 (n=5)	5.3 ± 1.7 (n=4)	7.6 ± 1.9 (n=7)
4-Day Survival Index[c]	46.4 ± 43.0[a] (n=5)	0[b] (n=4)	0[b] (n=7)

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c Four-day survival index = 100 × [number of pups viable at day 4 of age]/[number of viable pups born].

Table 12. Effects of TCB on body weights, organ weights, and relative organ weights of F-1 males at 3 weeks of age.

Weight[c]	Treatments		
	Control (n=7)	3 ppm (n=7)	30 ppm (n=8)
BW (g)	8.4 ± 0.5	8.7 ± 0.5	8.5 ± 0.7
LW (g)	0.35 ± 0.08	0.31 ± 0.05	0.34 ± 0.04
LW/BW × 100	4.2 ± 0.8	3.6 ± 0.5	4.0 ± 0.2
TMW (mg)	53.2 ± 5.0	53.3 ± 9.8	49.4 ± 13.4
TMW/BW × 100	0.64 ± 0.09	0.62 ± 0.13	0.58 ± 0.14
TTW (mg)	32.9 ± 3.3[a]	40.6 ± 5.7[ab]	43.2 ± 7.0[b]
TTW/BW × 100	0.39 ± 0.03[a]	0.47 ± 0.05[b]	0.51 ± 0.10[b]

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c BW, LW, TMW, TTW represent body, liver, thymus, and testis weights, respectively. Values represent mean organ weights or organ weights as percentages of body weights ± SD.

Table 13. Effects of TCB on body weights, organ weights, and relative organ weights of F-1 males at 9 weeks of age.

Weight[c]	Treatment		
	Control (n=10)	3 ppm (n=10)	30 ppm (n=10)
BW (g)	26.8 ± 2.2	23.5 ± 4.3	24.7 ± 2.5
LW (g)	1.25 ± 0.16[a]	1.26 ± 0.28[a]	1.48 ± 0.20[b]
LW/BW × 100	5.05 ± 0.50[a]	5.33 ± 0.45[a]	5.99 ± 0.46[b]
TMW (mg)	37.5 ± 13.1	32.5 ± 7.2	32.6 ± 5.0
TMW/BW × 100	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.01
TTW (mg)	194.3 ± 9.9	192.7 ± 10.8	197.6 ± 24.0
TTW/BW × 100	0.79 ± 0.06	0.85 ± 0.22	0.80 ± 0.08

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c BW, LW, TMW, TTW represent body, liver, thymus, and testis weights, respectively. Values represent mean organ weights or organ weights as percentages of body weights ± SD.

Table 14. Effects TCB on body weights, organ weights, and relative organ weights F-1 males at 19 weeks of age.

Weight[c]	Treatment		
	Control (n=6)	3 ppm (n=6)	30 ppm (n=6)
BW (g)	30.9 ± 2.1	29.4 ± 3.5	31.3 ± 2.0
LW (g)	1.32 ± 0.14[a]	1.32 ± 0.14[a]	1.67 ± 0.16[b]
LW/BW × 100	4.27 ± 0.31[a]	4.50 ± 0.21[a]	5.35 ± 0.31[b]
TMW (mg)	51.0 ± 11.8	38.0 ± 11.0	41.6 ± 12.0
TMW/BW × 100	0.16 ± 0.03	0.13 ± 0.03	0.14 ±0.04
TTW (mg)	219.4 ± 17.5	223.5 ± 17.8	232.4 ± 14.6
TTW/BW × 100	0.71 ± 0.05	0.77 ± 0.08	0.74 ± 0.04

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c BW, LW, TMW, TTW represent body, liver, thymus, and testis weights, respectively. Values represent mean organ weights or organ weights as percentages of body weights ± SD.

Table 15. Effects of TCB on reproductive performance of F-1 males at 7 weeks of age.

	Treatments		
	Control	3 ppm	30 ppm
No. Males Mated[a]	10	10	10
Fecundity (%)	90	80	90
Litter Size	8.9 ± 2.4 (n=9)	9.8 ± 2.4 (n=8)	9.3 ± 2.3 (n=9)
Sex Ratio (♂/♀)	2.0 ± 2.3 (n=9)	1.4 ± 0.8 (n=8)	1.3 ± 1.0 (n=9)
4-Day Survival Index[b]	100 (n=9)	100 (n=8)	100 (n=9)
21-Day Survival Index[c]	100 (n=9)	100 (n=8)	100 (n=9)

a One non-treated female was mated with one treated male for 10 days, from 7:30 PM to 7:30 AM. TCB treatment was withheld during breeding.

b Four-day survival index = 100 × [number of pups viable at day 4 of age]/[number of viable pups born].

c Twenty-one-day survival index = 100 × [number of pups viable at day 21 of age]/[number of pups retained at day 4 of age]. The litters in which all pups died before day 4 of age were not included.

Table 16. Effects of TCB on reproductive performance of F-1 males at 17 weeks of age.

	Treatments		
	Control	3 ppm	30 ppm
No. Males Mated[c]	6	6	6
Fecundity (%)	67	100	100
Litter Size	8.8 ± 1.3 (n=4)	9.0 ± 0.9 (n=6)	8.8 ± 1.2 (n=6)
Sex Ratio (♂/♀)	1.4 ± 1.0 (n=4)	2.4 ± 2.8 (n=6)	2.1 ± 2.9 (n=6)
4-Day Survival Index[d]	94.7 ± 6.1 (n=4)	100 (n=6)	100 (n=6)
21-Day Survival Index[e]	100 (n=4)	96.3 ± 9.1 (n=6)	100 (n=6)

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c One non-treated female was mated with 1 treated male for 10 days, from 7:30 PM to 7:30 AM. TCB treatment was withheld during breeding.

d Four-day survival index = 100 × [number of pups viable at day 4 of age]/[number of viable pups born].

e Twenty-one-day survival index = 100 × [number of pups viable at day 21 of age]/[number of pups retained at day 4 of age]. The litters in which all pups died before day 4 of age were not included.

Table 17. Effects of TCB on reproductive performance of F-1 males at 60 weeks of age.

	Treatments		
	Control	3 ppm	30 ppm
No. Males Mated[c]	5	4	5
Fecundity (%)	25	50	33
Litter Size	9.0 ± 1.0 (n=3)	8.0 ± 1.3 (n=6)	9.2 ± 2.2 (n=5)
Sex Ratio (♂/♀)	0.74 ± 0.13 (n=3)	1.54 ± 0.63 (n=6)	1.23 ± 0.70 (n=5)
4-Day Survival Index[d]	100[a] (n=3)	75.8 ± 28.3[b] (n=6)	78.5 ± 20.9[b] (n=5)
21-Day Survival Index[e]	100 (n=3)	100 (n=6)	100 (n=5)

ab Different superscripts represent significant differences within the same row ($P<0.05$).

c Three non-treated females were mated with 1 treated male for 90 days. TCB treatment was withheld during breeding.

d Four-day survival index = 100 × [number of pups viable at day 4 of age]/[number of viable pups born].

e Twenty-one-day survival index = 100 × [number of pups viable at day 21 of age]/[number of pups retained at day 4 of age]. The litters in which all pups died before day 4 of age were not counted.

Body weights of offspring sired by treated 7- and 60-week-old F-1 males were not affected (Tables 18 and 20). However, body weights of offspring sired by the 30 ppm-treated 17-week-old F-1 males were higher than the control (Table 19).

At 9 and 19 weeks of age, epididymal sperm was collected from treated F-1 males. Sperm concentration, velocity, linearity, ALH displacement, and beat/cross frequency were similar among all groups (Tables 21 and 22). Percentages of eggs fertilized by the sperm from 30 ppm-treated males was lower than the percentages for control group at 19 weeks of age, but not at 9 weeks of age (Tables 21 and 22). At 2.5 hours after sperm collection, decreased sperm motility was observed in 9-week-old 30 ppm-treated males. This was not observed at 0.25, 1.5, 3.5, and 4.5 hours after collection (Figure 1).

Table 18. Effects TCB on body weights of offspring sired by F-1 males at 7 weeks of age.

Age[a]		Treatment		
		Control	3 ppm	30 ppm
			BW (g)	
Day 4		2.4 ± 0.3 (n=9)	2.4 ± 0.3 (n=8)	2.3 ± 0.2 (n=9)
Week 1		4.3 ± 0.4 (n=9)	4.3 ± 0.4 (n=8)	4.2 ± 0.3 (n=9)
Week 2		8.2 ± 0.9 (n=9)	7.9 ± 0.9 (n=8)	7.9 ± 0.5 (n=9)
Week 3	(♂)	10.5 ± 1.0 (n=46)	10.3 ± 1.0 (n=42)	10.2 ± 0.9 (n=47)
	(♀)	10.5 ± 1.1 (n=23)	10.1 ± 1.0 (n=29)	10.0 ± 1.1 (n=36)

a Body weights on day 4, week 1, and week 2 represent the means ± SD of litters. Values for week 3 are means ± SD of individual weights.

Table 19. Effects of TCB on body weights of offspring sired by F-1 males at 17 weeks of age.

Age[a]		Treatments		
		Control	3 ppm	30 ppm
			BW (g)	
Day 4		2.0 ± 0.3 (n=4)	2.1 ± 0.1 (n=6)	2.5 ± 0.4 (n=6)
Week 1		3.7 ± 0.4 (n=4)	4.0 ± 0.2 (n=6)	4.4 ± 0.5 (n=6)
Week 2		7.6 ± 0.7 (n=4)	7.8 ± 0.9 (n=6)	8.0 ± 0.7 (n=6)
Week 3	(♂)	9.8 ± 1.6[a] (n=17)	10.5 ± 1.1[ab] (n=30)	10.7 ± 1.0[b] (n=27)
	(♀)	9.4 ± 1.2[a] (n=16)	10.2 ± 0.9[b] (n=22)	10.5 ± 1.0[b] (n=25)

a Body weights on day 4, week 1, and week 2 represent the means ± SD of litters. Values for week 3 are means ± SD of individual weights.

Table 20. Effects of TCB on body weights of offspring sired by F-1 males at 60 weeks of age.

Age[b]		Treatments[a]		
		Control	3 ppm	30 ppm
		BW (g)		
Week 1		4.0 ± 0.3 (n=3)	3.5 ± 0.6 (n=6)	3.5 ± 1.0 (n=4)
Week 2		7.8 ± 0.7 (n=3)	7.4 ± 1.3 (n=6)	8.0 ± 1.5 (n=4)
Week 3	(♂)	10.2 ± 1.2 (n=12)	10.3 ± 1.3 (n=20)	10.5 ± 1.6 (n=17)
	(♀)	10.2 ± 1.2 (n=16)	10.0 ± 1.9 (n=12)	9.7 ± 1.1 (n=13)

a Three non-treated females were mated with 1 treated male for 90 days. TCB treatment was withheld during breeding.

b Body weights on week 1 and week 2 represent the means ± SD of litters. Values for week 3 are means ± SD of individual weights.

Table 21. Effects of TCB on fertilization rates *in vitro* and sperm motion analysis for F-1 males at 9 weeks of age.

Parameters[a]	Treatments		
	Control (n=10)	3 ppm (n=10)	30 ppm (n=10)
Concentration ($\times 10^6$/ml)	12.4 ± 3.1	12.7 ± 5.8	12.1 ± 3.8
Motility (%)[b]	70.9 ± 10.2	69.2 ± 11.7	72.9 ± 7.0
Velocity (μm/sec)[c]	246.9 ± 59.9	210.8 ± 92.9	260.2 ± 67.3
Linearity[d]	5.4 ± 0.8	4.6 ± 1.8	5.6 ± 0.9
Amplitude of Lateral Head Displacement (μm)[e]	7.3 ± 1.9	5.9 ± 2.8	7.6 ± 3.4
Beat/Cross Freq. (Hz)[f]	12.7 ± 1.3	11.2 ± 4.3	12.6 ± 1.6
Fertilization *In Vitro* (%)	66.1 ± 27.4	68.9 ± 28.1	63.3 ± 21.6

a *In vitro* fertilization and sperm motion analysis were performed 1.5 hours after sperm collection.

b Percentage of sperm that travel more than 20 μm/sec.

c Average distance traveled by motile sperm.

d A calculation of the length of the straight line distance divided by the track distance covered by sperm, multipled by 10. This is expressed on a scale of 0 to 10; 10 indicates a perfectly straight line and 0 indicates a circular track.

e A measure of the displacement of the sperm head from a computer-calculated curval mean of its track.

f The number of beats (or crosses) per second. Every time the sperm cell crosses the computer-calculated curval mean, the computer counts that crossing as one beat.

Table 22. Effects of TCB on fertilization rates *in vitro* and sperm motion analysis for F-1 males at 19 weeks of age.

Parameters[c]	Treatments		
	Control (n=6)	3 ppm (n=6)	30 ppm (n=6)
Concentration ($\times 10^6$/ml)	13.6 ± 2.8	11.6 ± 3.3	11.0 ± 2.0
Motility (%)[d]	63.5 ± 9.5	60.6 ± 13.2	66.5 ± 10.3
Velocity (μm/sec)[e]	208.9 ± 35.4	190.8 ± 24.0	200.9 ± 11.8
Linearity[f]	4.9 ± 0.2	4.8 ± 1.2	4.7 ± 0.5
Amplitude of Lateral Head Displacement (μm)[g]	5.7 ± 1.5	5.5 ± 1.1	5.5 ± 1.9
Beat/Cross Freq. (Hz)[h]	11.5 ± 1.9	12.7 ± 2.3	12.6 ± 1.0
Fertilization *In Vitro* (%)	57.9 ± 12.6[a]	57.2 ± 19.7[a]	46.7 ± 11.5[b]

ab Different superscripts represent significant differences within the same row.

c *In vitro* fertilization and sperm motion analysis were performed 1.5 hours after sperm collection.

d Percentage of sperm that travel more than 20 μm/sec.

e Average distance traveled by motile sperm.

f A calculation of the length of the straight line distance divided by the track distance covered by sperm, multipled by 10. This is expressed on a scale of 0 to 10; 10 indicates a perfectly straight line and 0 indicates a circular track.

g A measure of the displacement of the sperm head from a computer-calculated curval mean of its track.

h The number of beats (or crosses) per second. Every time the sperm cell crosses the computer-calculated curval mean, the computer counts that crossing as one beat.

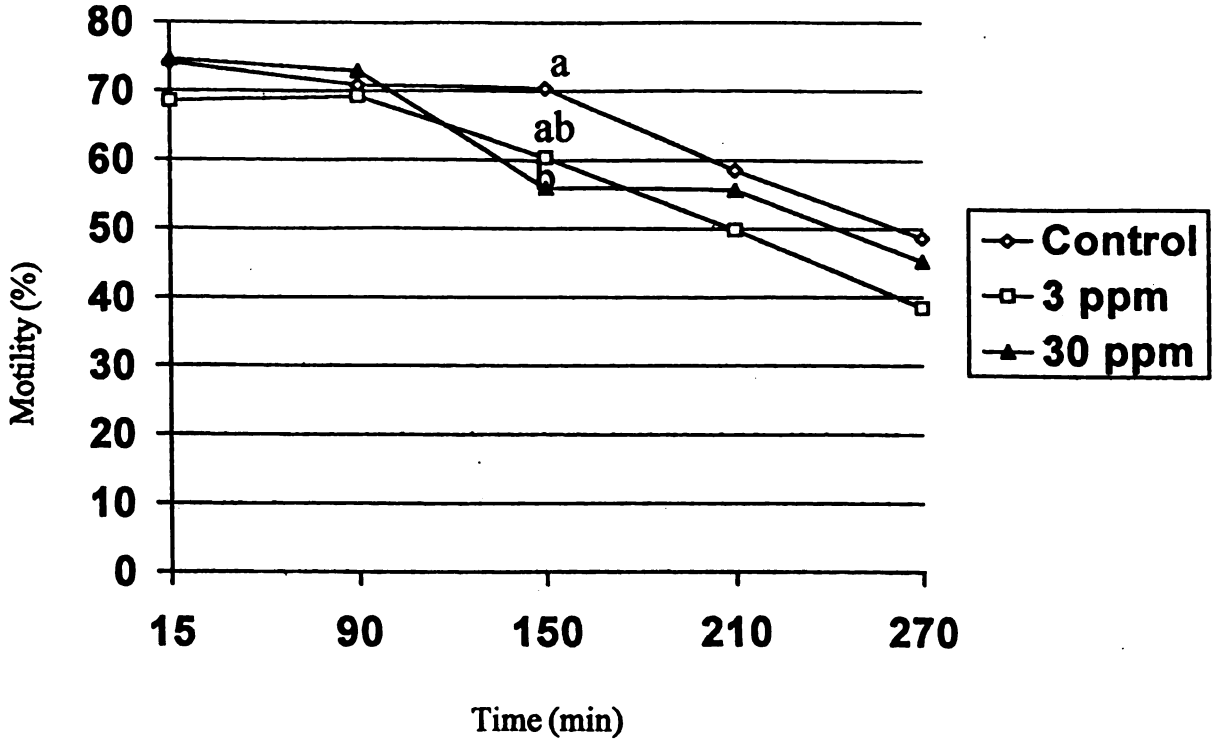

Figure 1. Motility of sperm taken from 9-week-old F-1 males. Females (F-0) were treated with 0, 3, and 30 ppm TCB 2 weeks prior to breeding. Treatments continued through mating, gestation, and lactation. Offspring (F-1) were given the same diet as their dams received. Each value reflects the mean of data from 7 mice. Different superscripts represent significant differences ($P<0.05$).

DISCUSSION

Phase I

Results of this study demonstrated that accumulation of TCB in liver and adipose tissue is not arithmetically proportional to exposure concentrations. After 5 weeks of exposure, concentrations of TCB equivalents in the liver, adipose tissue, uterus, and fetus of mice in the 30 ppm group were only 3-6 times higher than concentrations in mice in the 3 ppm group. Greater induction of hepatic microsomal monooxygenases has been observed in pregnant rabbits treated with 10 mg Aroclor 1254/kg bw than in those treated with 1.0 mg/kg (Villeneuve and Grant, 1971). Thus, it is possible that in the present study, hepatic microsomal monooxygenases in the 30 ppm-treated mice were also induced to a greater extent than in 3 ppm-treated mice, resulting in an increase in metabolism and elimination of TCB in the high dose group.

After 5 weeks, the concentrations of TCB equivalents in the liver of pregnant mice treated with 30 ppm TCB in the feed was 1.8 times higher than those in non-pregnant mice. This may be due, in part, to an increase in the amount of fat in the liver during pregnancy, causing a redistribution of TCB and its metabolites from adipose tissues to liver. This hypothesis is also evident in the low concentrations of TCB equivalents in the adipose tissue of pregnant mice, which was 2/3 of that in non-pregnant mice. Increases in deposits of body fat cause dilution of TCB concentrations and may

also contribute to lower concentrations of TCB equivalents in pregnant mice than in the non-pregnant mice. Nonetheless, when two groups of mice were exposed to a single injection of 100 mg ^{14}C-6-CB/kg bw, and one group was then mated while one group remained virgin, radioactivity in the kidney, adipose tissue, and liver of pregnant mice was higher than that in the virgins sacrificed concurrently (Vodicnik and Lech, 1980). The differences in adipose tissue radioactivity in the two studies could be attributed to different administration doses and different congeners. Induction of hepatic microsomal ethoxycoumarin-*O*-deethylase (ECOD) by 4-CB was less in pregnant mice than in virgins over a 4-day period (Vodicnik, 1986). A slower rate of elimination of 4-CB equivalents was observed in pregnant mice than in virgin mice.

Results in the present study also indicated that dosage affected distribution of TCB in the liver and adipose tissue. The concentrations of TCB equivalents in the liver and adipose tissue of the 3 ppm-treated mice were the same in pregnant and non-pregnant mice. Any differences in body burden between pregnant and non-pregnant mice can not be explained, since PCBs in the fetus only account for approximately 0.003% of maternal body burden of pregnant rats (Takagi *et al.*, 1976).

In this study, concentrations of TCB equivalents, calculated from radioactivity, were similar in the uteri, placentas, and fetuses of treated mice. Darnerud *et al.* (1986) reported that TCB metabolites, but no parent TCB, were detected in the fetus when pregnant mice were gavaged with 25 mg TCB/kg bw on day 13 of gestation and sacrificed on day 17 of gestation. Therefore, it is likely that the radioactivity detected in the fetal tissue in the current study is from TCB metabolites.

Results from this study also point to redistribution of TCB equivalents to the thymus from other tissues during lactation in the 30 ppm group. Although ^{14}C-TCB feed was changed to TCB feed at parturition, radioactivity in the thymus increased during lactation. However, only background radioactivity values were detected in the thymus of the 3 ppm-treated mice. Although TCB redistribution into the thymus was observed in the high dose group during lactation, the mechanisms by which this occurs is unknown.

The redistribution of radioactivity in pregnant and lactating mice is likely to be associated with the increase of very low density lipoprotein (VLDL), the primary carrier of 6-CB *in vivo* which occurs during gestation (Spindler-Vomachka and Vodicnik, 1984). VLDL is a major substrate for mammary gland lipoprotein lipase which is elevated during late gestation and lactation (Spindler-Vomachka and Vodicnik, 1984). More than 70% of circulating 6-CB was associated with VLDL during late gestation in female mice treated with 6-CB two weeks prior to mating (Gallenberg and Vodicnik, 1987). Continuous exposure to varying doses of TCB results in differences in TCB redistribution between organs and tissues. In the 3 ppm-treated group, approximately 85% of the concentrations of TCB equivalents disappeared from both liver and adipose tissue during lactation. However, in the 30 ppm group, 96% disappeared from the liver and only 44% disappeared from the adipose tissue. It is possible that circulating lipoproteins in the blood were "saturated" with TCB equivalents in the 30 ppm group, which decreased the redistribution of TCB from adipose tissue to liver. This phenomenon,"saturable kinetics", was observed in the fetal compartment when dams were pretreated with TCB

(12.5-50 mg/kg BW) and followed 4 hours later by ^{14}C-TCB (10 mg/kg) (Darnerud *et al.*, 1986). Radioactivity in fetal brain, liver, thymus, skin, and muscle in the pretreatment groups was less than half of that observed in the group without TCB pretreatment (Darnerud *et al.*, 1986).

Different doses affect the distribution of TCB in the liver and adipose tissue of offspring. In the offspring of the 30 ppm treatment group, concentrations of TCB equivalents in the adipose tissue were 14.6 times higher than those in the liver. However, concentrations of TCB equivalents in the adipose tissue were only 1.5 times higher than those in the liver of 3 ppm-exposed offspring. In addition, although the pups were nursed by the same dam in the 30 ppm group, large variations in concentrations of TCB equivalents were observed. These results are due to large variations of offspring body weights and fat deposits in the 30 ppm-exposed offspring. Variations of offspring body weights were smaller in the 3 ppm group than those in the 30 ppm group.

This preliminary study indicates that accumulation of TCB equivalents in the adipose tissue and liver of mice is not arithmetically proportional to exposure. This is probably due to the greater induction of hepatic microsomal monooxygenases in 30 ppm-treated mice than in the 3 ppm-treated mice, resulting in an increase in metabolism and excretion of TCB. A similar accumulation of TCB equivalents between 3 ppm-treated non-pregnant and pregnant mice suggests similar activities of hepatic microsomal monooxygenases under differing physiological conditions. Different doses result in variations of TCB redistribution during late gestation and lactation.

Phase II

Feed Intake and Body Weight

Based on feed intake, F-0 females in the 30 ppm treatment group consumed approximately 7.5, 5.9, and 8.3 mg TCB/kg bw/day before breeding, from day 1 to day 16 of gestation, and during lactation, respectively. Body weights were not significantly different among F-0 treatment groups before breeding and during gestation. During lactation, body weights in the 30 ppm group were higher than those in the 3 ppm group. However, body weights in the control group were not different from TCB treated groups. As Yoshimura *et al.* (1979) reported, body weight gains in weanling rats over a 4-day period were not affected by a single i.p. injection of 50 mg TCB/kg bw. In immature male rats, ED50 for body weight loss caused by TCB was approximately 213 mg/kg bw in a single i.p. injection (Leece *et al.*, 1985). Reduced body weight gains were reported by Sanders *et al.* (1974) in rats after 2 weeks of 62.5 or 1000 ppm of Aroclor 1254 exposure through the feed. These doses are much higher than the high dose tested in this study which was 7.5 mg/kg bw/day prior to breeding. High feed intake observed in the 3 ppm-treated mice during lactation was in all likelihood a compensation response to the low feed intake before breeding and during gestation.

Changes in body weights of offspring treated with PCBs perinatally were dependent on exposure period, dose, and on the congener or commercial mixture. F-1 body weights at weaning in the 3 ppm group were numerically greater than those in the 30 ppm group. The increase in F-1 weaning weights in the 3 ppm group could be the result of increase in milk production of the dams which had high feed intake. As Gellert

and Wilson (1979) reported, no effects on body weights of female rat offspring were observed when dams were gavaged with 30 mg Aroclors 1242 and 1260/kg bw daily on days 14-20 of gestation. Offspring body weights, however, increased when dams were treated with Aroclor 1221 (Gellert and Wilson, 1979). On the other hand, decreased body weights of young mice were observed when parents were dosed with 10 mg Aroclor 1254/kg bw throughout gestation and lactation (Linzey, 1988), although these litters did not differ in body weights at birth. Similar regimens using different PCBs and doses produced diverse results. The relative growth rates of young male mice from dams administered orally 0.005 mg 2,4',5-trichlorobiphenyl or 6-CB/day, from day 5 of gestation to day 22 of lactation, were higher than those of male offspring in the control group (Orberg, 1978b). The differences in offspring body weights at weaning are mostly diminished after maturity. For example, decreased body weight gains during lactation followed by increased body weight gains after weaning were observed in rat offspring when dams were treated on days 1, 3, 5, 7, and 9 postpartum with a dose of 32 or 64 mg Aroclor 1254/kg bw (Sager *et al.*, 1991). Weight gains were similar among treatment groups after those offspring reached 5 months of age.

In the present study, the body weights of offspring of treated F-1 males are dependent on the exposure period. No differences were observed in body weights of offspring born to non-treated females bred with 7-week-old 30 ppm-treated F-1 males. However, body weights of offspring from non-treated females bred with 17-week-old 30 ppm-treated F-1 males increased compared to control. These increases occurred at 3 weeks of age and were independent of the body weights of treated F-1 males and dams

at weaning. Since only sperm from the treated males could have contributed to the increases in body weights,TCB exposure must have altered some characteristic of pre-ejaculated sperm.

Hepatotoxicity

Liver enlargement has been observed by others in TCB-treated rats (Yoshimura *et al.*, 1979; Clarke *et al.*, 1984). Increased numbers of peroxisomes and lipid droplets, proliferated smooth endoplasmic reticulum, abnormal mitochondria (Harris and Bradshaw, 1984; MacLellan *et al.*, 1994), and distended cisternae of the rough endoplasmic reticulum (Harris and Bradshaw, 1984) are the most overt signs of toxicity in the livers of rats treated with TCB. Total liver lipid content, including cholesterol, phospholipid and neutral lipid, increased in mature rats exposed to 50 or 500 ppm Aroclor 1254 for 3 weeks (Garthoff *et al.*, 1977). Total protein was also increased by the 500 ppm treatment, while the relative total protein (mg/g wet weight) decreased. The increases in lipid content were associated with increases in the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme of cholesterol synthesis, as well as increases in three NADPH-generating enzymes: malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase (Kato and Yoshida, 1980; Hitomi *et al.*, 1993). NADPH is essential for biosynthesis of cholesterol, lipid, and several amino acids (Voet and Voet, 1990).

This study demonstrated that the effects of TCB on liver weights depends on the physiological condition and age of the animal. Increases in liver weights were apparent in F-0 pregnant and lactating mice, but not in non-pregnant mice. Large variations in

liver weights of non-pregnant mice may contribute to the lack of statistical significance of the data. Notably, F-1 females treated with 30 ppm TCB showed increases in liver weights at 5 and 6 weeks of age, but not in relative liver weights. At 7 weeks of age, while there was an increase in liver weights and relative liver weights in the 30 ppm group, the differences were not statistically significant.

Differences in exposure period and inducibility of hepatic microsomal enzymes may have contributed to the differences in offspring liver weights before and after puberty. Both liver weights and relative liver weights increased in the 30 ppm group in F-1 males at both 9 and 19 weeks of age, but not at 3 weeks of age. A significant induction of uridine diphosphoglucuronic acid glucuronyltransferase (UDPGT), an important microsomal glucuronide conjugation enzyme, was observed in the TCB treated rats (3 mg/kg bw/day prenatally) at 3 weeks of age (Harris and Bradshaw, 1984). However, this induction diminished after 3 weeks of age. In addition, a higher-fold induction of hepatic microsomal monooxygenases above the control level was observed in the weanlings than in the adults (Chen and DuBois, 1973). This high induction of hepatic microsomal enzymes enhances biotransformation and excretion of TCB in males at 3 weeks of age.

Thymic Atrophy

Thymic atrophy is a consistently observed effect of TCDD and PCBs in all animals thus far examined (Faith and Moore, 1977; McConnell, 1989; Smialowicz *et al.*, 1989). Microscopically, the reduction in size is reflected almost entirely as a loss of cortical lymphocytes (McConnell *et al.*, 1978). Although Clarke *et al.* (1984) reported

that 5 mg TCB/kg bw/day for 3 weeks caused thymic atrophy in young female rats, the present study showed no such changes in F-0 female mice after 5 weeks of treatment. The difference between the two observations may be explained by species variation or the differences in maturity of the test animals. Rats in the study by Clarke *et al.* weighed only half of their mature body weight, while the F-0 females in the present study had reached their mature body weight. Decreases in thymus/body weight ratios in the offspring of dams exposed to TCDD during gestation and lactation were more apparent than those only exposed to TCDD postnatally (Faith and Moore, 1977). At 5, 6 and 7 weeks of age, F-1 females treated with 30 ppm TCB showed decreases in thymus weights, however, TCB did not induce thymic atrophy in F-1 males at 3, 9, and 19 weeks of age in the present study. Gender appears to be the primary factor contributing to the differences of thymic effects between the F-1 males and females.

Female Reproductive Toxicity

In our study, fecundity in 30 ppm-exposed females (F-0) was reduced by 40%, while the fecundity in treated F-1 females was the same as in the control group. The decrease in the F-0 females could be the result of many reproductive dysfunctions from mating to fertilization, to embryo and fetal development. Existing information points to a decrease in mating of PCB-treated females. In rats, the percent of females mated, judged by vaginal plugs, decreased by 40% when those females were dosed with 7.5 mg Aroclor 1242/kg bw/day for 36 weeks (Jonsson *et al.*, 1976). Similar results have been reported by Brezner *et al.* (1984) who stated that sexual receptivity was decreased by 20% in rats exposed orally to 30 mg Aroclor 1254/kg bw for 1 month. This sexual

receptivity was determined by the presence of sperm in the vaginal smear on the day after mating. Small sample sizes of F-1 females may, in part, have prevented the detection of differences in fecundity among treatment groups.

Mortality of offspring mostly occurred within 4 days postpartum. Litter sizes of treated F-0 females and F-1 offspring were similar among all 3 groups at parturition. These results reflect the observations by Rands *et al.* (1982) that the mortality during parturition was the same in TCB treated rats as in controls. Four-day and 21-day survival indices only decreased in the 30 ppm group in F-0 females, while all pups born to TCB treated F-1 females of both dose groups died within 4 days of age. In rats, a high incidence of neonatal mortality was also observed after prenatal exposure to TCB at 3 mg/kg bw/day from day 6 through day 18 of gestation (Rands *et al.*, 1982; Harris and Bradshaw, 1984). The reasons for the mortality of the progeny are likely associated with the behavioral changes of dams (Pantaleoni *et al.*, 1988), or direct toxicity caused by TCB and its metabolites (Darnerud *et al.*, 1986), or some combination of these factors. In addition, there is a low percentage of fat in the young which is one of the major tissues where PCBs accumulate. This heightens the amount of absorbed PCBs distributed to target organs and tissues in the body.

Postnatal mortality is also related to toxicity of TCB and its metabolites in embryos and fetuses. Accumulations of 2-hydroxy-TCB and methylsulphonyl-tetrachlorobiphenyl, two TCB metabolites, in fetal tissue and uterine fluid were observed after a single intravenous injection of 3.5 mg TCB/kg bw in pregnant mice (Darnerud *et al.*, 1986). Hemorrhage in intestinal mucosa of fetuses was observed when pregnant

rats were treated with 3 mg TCB/kg bw on days 6-18 of gestation (Rands *et al.*, 1982). Embryonic death in mice increased after a single oral dose of 25 mg TCB/kg bw on day 11 of gestation (d'Argy *et al.*, 1987). Since the parent TCB was not found in fetal tissue (Darnerud *et al.*, 1986), the metabolites of TCB play a very important role in embryotoxicity.

The *in vitro* fertilization assay showed that egg fertilizing ability decreased in F-1 females exposed to 3 ppm and 30 ppm TCB. The poor egg quality was also demonstrated by the high percentage of degenerated eggs after collection and culture. Similar results were observed in a study in which sperm and eggs were incubated in medium containing PCBs. After 1.5 hours of incubation, sperm were added to the petri dish where superovulated eggs were treated with the following PCBs: TCB, Aroclor 1221, and Aroclor 1268. Fertilization rates decreased at a concentration of 1 μg/ml for these PCBs tested (Kholkute *et al.*, 1994).

In the present study, it is suggested that impaired egg quality is associated with decreased estrogen levels in the follicles. Preovulatory follicular growth is dependent on the interaction of estradiol, FSH, and LH (Richards, 1980). The ability of healthy eggs with germinal vesicles to resume meiosis *in vitro* is highly correlated with the concentration of estradiol in antral fluid (McNatty *et al.*, 1979). Theca interna, an androgen biosynthesizing site in the ovary in all species, is differentiated from stroma cells. The synthesized androgens are then transferred to the basement membrane of the follicle and on to the granulosas where they are aromatized to estrogens (Peters, 1979; Erickson *et al.*, 1985; Ryan, 1988). Characteristic changes in the ovarian stroma cells,

including a spindling of ovarian cells accompanied by looseness of cellular arrangement and reduction of follicle numbers, were observed in 150 ppm Aroclor 1242-treated rats (Jonsson *et al.*, 1976). The stroma changes may affect differentiation of theca interna and decrease androgen and estrogen biosynthesis. In addition, metabolism and elimination of estrogen can be increased by hepatic microsomal monooxygenases induced by PCBs or other chemicals (e.g., phenobarbital) (Risebrough and Brodine, 1970; Chen *et al.*, 1993).

Environmental estrogens, including PCBs, have received much attention lately (Hileman, 1994; Stone, 1994). Several commercial PCB mixtures and congeners related to estrogenic activity have been investigated (Bitman and Cecil, 1970; Ecobichon and MacKenzie, 1974; Gellert, 1978; Korach *et al.*, 1987). In general, the lesser chlorinated PCBs have higher estrogenic activity than the more highly chlorinated PCBs, based on the glycogen response of the immature rat uterus. It has been suggested that uterotropic activity of PCBs is dependent upon the formation of metabolites (Ecobichon and MacKenzie, 1974). The lesser chlorinated PCBs can be more easily biotransformed to hydroxylated metabolites than the more highly chlorinated PCBs. In addition, for the hydroxylated congeners tested, those compounds with strong affinities to estrogen receptors possess either single or multiple *ortho*-chlorine substitutions (Korach *et al.*, 1987). No *ortho*-substituted TCB metabolites were detected in rats (Yoshimura and Yamamoto, 1974; Yoshimura *et al.*, 1987; Koga *et al.*, 1989) and mice (Darnerud *et al.*, 1986; Klasson Wehler *et al.*, 1989). Although TCB metabolites show relatively little estrogenic activity, the role they play in reproductive and developmental toxicity can not

be ignored.

In the present study, eggs superovulated by exogenous PMSG and HCG were similar in all groups. Similar results were observed by Brezner *et al.* (1984) in that the number of ovulations was not affected in mature rats exposed to Aroclor 1254 at a dose of 10 mg/kg bw/day for 1 month. In contrast, both decreases and increases in germ cells have been reported in TCB-exposed mouse offspring (Ronnback, 1991; Ronnback and de Rooij, 1994). The different results appear to be dependent on exposure regimens in the 2 studies. F-0 females in the Ronnback (1991) study were only injected intraperitoneally on day 13 of gestation with 1.5-15 mg TCB/kg bw. However, in the study by Ronnback and de Rooij (1994), F-0 females were gavaged with TCB at 9 or 15 mg TCB/kg bw weekly for 2 weeks prior to mating. The treatment continued through gestation and lactation for a total of 7 doses. The increases in germ cells in F-1 females were associated with the inhibition of atresia (Ronnback and de Rooij, 1994).

Male Reproductive Toxicity

In this study, testis weights increased in F-1 males at 3 weeks of age, but not at 9 and 19 weeks of age. Similar results were also observed in albino mice (Johansson, 1987). Increases in relative testis weights (mg/100 g bw) were noted in mice treated perinatally with 6-CB at 200 or 400 mg/kg bw doses, but not in mice treated in the pubertal period (Johansson, 1987). Testis weights were not affected in studies where adult rats were exposed to 500 ppm Aroclor 1254 (Garthoff *et al.*, 1977) or in adult mice treated with 200 or 1000 ppm Aroclor 1254 (Sanders *et al.*, 1974, 1977). In contrast, increased testis weights of offspring were observed in pubertal rats when dams were

treated with 32 mg Aroclor 1254/kg bw on days 1, 3, 5, 7, and 9 of lactation (Sager *et al.*, 1983).

Existing knowledge suggests two possible mechanisms for these effects. One is the development of a blood-testis barrier which may play a role in decreasing testis toxicity in adults. The blood-testis barrier develops at the time of puberty and just prior to the onset of spermatogenesis (Sever and Hessol, 1985; Thomas, 1991). In rats, the barriers between pairs of Sertoli cells form at approximately 18 days of age (Gilula *et al.*, 1976). A second possible mechanism is the induction of hepatic microsomal enzymes. Induction of *N*-demethylase in treated weanling rats was 3.5-4.2 times that of the control group, while treated adults had induction of 2.4-2.8 times that of control group. This pattern also exists with *O*-ethyl *O*-(4-nitrophenyl)phenyl phosphonothioate (EPN) detoxification activity (Chen and DuBois, 1973). Uridine diphosphoglucuronic acid glucuronyltransferase (UDPGT), one of the major enzymes in the second phase of biotransformation, converts both exogenous and endogenous compounds to polar and water-soluble compounds (Sipes and Gandolfi, 1991). When pregnant rats were orally administered 3 mg TCB/kg bw daily on days 8 - 18 of gestation, the hepatic UDPGT activity in the offspring increased at 3 weeks of age, but diminished by 8 weeks of age (Lucier and McDaniel, 1979). The increased induction of hepatic enzymes may enhance the metabolism of testosterone in weanlings. The enlargement of the testis may have been compensation for the enhanced testosterone metabolism by PCB-induced hepatic microsomal enzymes (Risebrough and Brodine, 1970; Haake-McMillan and Safe, 1991).

Testicular toxicity may result in impairment of spermatogenesis. In this study,

epididymal sperm concentration was similar among the treatment groups at both 9 and 19 weeks of age. To the contrary, Sanders *et al.* (1977) reported that 200 ppm Aroclor 1254 in the feed of adult albino male mice significantly reduced the number of sperm cells per milligram of testis. A similar effect of Aroclor 1254 was observed in adult white-footed male mice (Sanders and Kirkpatrick, 1975). Exposure to 400 ppm Aroclor 1254 for 2 weeks reduced the number of sperm per testis. There was no effect on the number of sperm cells at 100 or 200 ppm (Sanders and Kirkpatrick, 1975). Compared with those observations, the doses which affected sperm production were much higher than the high dose in the present study. The sites used to measure the number of sperm may have led to the diversity of results. The authors measured the number of sperm in the testis (Sanders and Kirkpatrick, 1975; Sanders *et al.*, 1977), however, in the present study, the epididymal sperm concentration was measured. Although enlarged testes were observed at 3 weeks of age, epididymal sperm concentration was not affected when mice were at 9 and 19 weeks.

Decreases in egg fertilizing ability *in vitro* were observed with sperm from 30 ppm-treated F-1 males at 19 weeks of age, but not at 9 weeks of age. In another study, a decrease in the normal number of embryos was observed as a result of the mating of non-treated female rats with treated males dosed with 8 mg Aroclor 1254/kg bw on days 1, 3, 5, 7, and 9 of lactation (Sager *et al.*, 1987, 1991). Based on the existing information, there are two possible mechanisms associated with this decreased sperm quality. The first one is testicular oxidative stress. Two of the antioxidant enzymes, superoxide dismutase (SOD) and catalase, decreased in mature rats after a single i.p.

injection of 100 mg Clophen A 50/kg bw (Peltola *et al.*, 1994). In addition, testicular mRNAs for NADPH-generating enzymes decreased by 23-32% in rats fed 200 ppm Aroclor 1254 for 3 days (Hitomi *et al.*, 1993). NADPH, generated primarily by the reactions catalyzed by malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PD), and 6-phosphogluconate dehydrogenase (6PGD) (Voet and Voet, 1990), is necessary for the reduction of glutathione disulfide to glutathione. Secondly, administration of TCB throughout gestation and lactation is likely to disrupt hormonal balance during postnatal development, thus affecting spermatogenesis. Decreases in plasma testosterone were observed in 5- and 10-week-old male offspring of dams gavaged with 3 mg TCB/kg bw/day on day 6 through 18 of gestation (Vincent *et al.*, 1992).

Stem cells differentiate into type A spermatogonia during early postpartum. The number of type A spermatogonia continues to increase until 35 days of age, and decreases thereafter in mice (Foster, 1989; Vergouwen *et al.*, 1993). Rapidly dividing and differentiating spermatogonia are the cells most susceptible to chemical toxicity during spermatogenesis (Meistrich, 1986). Sensitivity of stem cells to toxic materials in this critical period explains, in part, the affected sperm quality in this study and in those studies by Sager *et al.* (1987 and 1991). Exposure during the critical postnatal period (approximately one week) may affect subsequent reproductive performance. In the Sager studies, lactating dams were treated orally on days 1, 3, 5, 7, and 9 postpartum. Reduced incidences of implantation were observed in non-treated females mated with treated males. The lowest observable adverse effect level was the maternal oral dose of 8 mg Aroclor 1254/kg bw every 2 days during days 1-9 of lactation. However, no effect

on fertility was observed by Kihlstrom *et al.* (1975) when male mice offspring exposed to PCBs during suckling were mated to non-treated females. In that study, those offspring were exposed to PCBs only from dams injected with 50 mg Clophen A 60/kg bw subcutaneously on the day of parturition and repeated weekly for three successive weeks (Kihlstrom *et al.*, 1975). The frequency of administration during the critical postnatal period in the Sager studies is higher than that in the study by Kihlstrom *et al.* (1975). This may account for their different results.

Parameters assessed by sperm motion analysis were similar among all treatment groups, except for the decreased motility observed in the 30 ppm-treated males at 2.5 hours after collection. These parameters included velocity, linearity, ALH displacement, beat/cross frequency, and motility. The decreased sperm motility was probably associated with inhibition of ATPase inhibition (La Rocca and Carlson, 1979). A significant decrease in total ATPase activity of liver, kidney, and brain tissue was observed in rats treated with 25 mg Aroclor 1242/kg bw for 7 days (La Rocca and Carlson, 1979). It is well established that ATPase causes the hydrolysis of ATP to ADP with the release of energy. This is critical for sperm motility. Fecundity and litter sizes were the same in non-treated females bred with treated F-1 males at 7 and 17 weeks of age. The 21-day survival index was decreased in the offspring of treated F-1 males at 60 weeks of age in the 3 ppm and 30 ppm groups. Sperm from the treated males was the only factor resulting in decreased survival of offspring. It is, therefore, hypothesized that TCB exposure must have altered some characteristic of sperm. This conclusion should be taken conservatively due to the small sample sizes in this observation. Only

1-2 males in each treatment group (4-5 males/group) at 60 weeks of age were fertile after 3 months of breeding with non-treated females.

Sexual Differentiation

Androgenic deficiency caused by exposure to TCDD and PCBs during prenatal and early postnatal periods can impair male reproductive function by disrupting the development of sex organs and impairing sexual differentiation of the central nervous system. Hormones regulate sexual differentiation in the critical periods during both prenatal and early postnatal stages (George and Wilson, 1988; Hadley 1992). Testosterone and DHT are responsible for the differentiation of male internal genital ducts and adult secondary sex characteristics, and external genitalia, respectively (Hadley, 1992). When testosterone in the brain is transformed into estradiol by aromatase, the estradiol controls the differentiation of male hypothalamus (Hadley 1992).

Sex ratios were similar throughout this study in both treated males and females. In contrast, the sex ratios (♂/♀) of neonates decreased when pregnant rats were treated by gavage with a single dose of 3 mg TCB/kg bw from day 6 to day 18 of gestation (the exact day was not reported) (Simmons *et al.*, 1984). The high dose used in the present study was approximately 7.5 and 5.9 mg TCB/kg bw/day before breeding and from day 1 to day 16 of gestation, respectively. It is likely that the induction of hepatic microsomal monooxygenases biotransformed the highly toxic TCB into less toxic metabolites when dams were treated continuously in the present study. However, in the study by Simmons *et al.*, TCB was administered during the critical period when organogenesis occurred. Large variations of sex ratios were observed in all groups

which made it difficult to establish a statistical difference in sex ratios among treatment groups. A decrease in testosterone concentrations was observed in the fetus on days 18 through 21, and shortly after birth when dams were treated with 1.0 μg TCDD/kg bw on day 15 of gestation (Mably *et al.*, 1992). Anogenital distances, measured 1-5 days after parturition, were shorter in the TCDD exposed neonates than those in the controls. Thus, TCDD and PCBs may cause feminization by disrupting the hormonal balance, especially that of testosterone and DHT.

CONCLUSIONS

Liver weights increased in the 30 ppm-treated lactating F-0 mice and in all F-1 mice except for the weanling males. Exposure period and induction of hepatic microsomal enzymes may explain the differences of liver weights between F-1 male weanlings and adults. Thymus weights only decreased in the F-1 females between 5 and 7 weeks of age. In this study, thymic effects were shown to be age and sex dependent.

Fecundity only decreased in the 30 ppm-treated F-0 group, but not in the F-1 mice. Unaffected fecundity in F-1 mice can be attributed to small sample sizes. Survival of the offspring of treated F-0 and F-1 females decreased. This increased mortality is probably associated with either the behavioral changes of dams or direct toxicity caused by TCB and its metabolites, or any combination of the two factors. Increased testis weights were only observed in the F-1 weanlings, not in the pubertal and mature mice. Development of blood-testis junction and hepatic microsomal enzymes may contribute to this result. Decreased gamete fertilizing ability was observed in both treated F-1 males and females.

Reproductive performance and gamete integrity were impaired by TCB exposure. Since testosterone, DHT, and estrogen are critical for organogenesis and gametogenesis, these effects are in all likelihood predominantly associated with the hormonal imbalance caused by TCB treatment.

APPENDIX

APPENDIX I

Appendix I. Specific activity of ^{14}C-TCB in feed

1 kg of 3 ppm TCB feed
Specific activity of ^{14}C-TCB
= ^{14}C-TCB/total TCB
= 9.52 μCi/3 mg TCB
= 3.17 μCi/mg TCB

1 kg of 30 ppm TCB feed
Specific activity of ^{14}C-TCB= ^{14}C-TCB/total TCB
= 9.52 μCi/30 mg TCB
= 0.317 μCi/mg TCB

APPENDIX II

Appendix II. Calculation of concentrations of TCB equivalents in the sample.

DPM
= CPM/efficiency of liquid scintillation counter for ^{14}C
= CPM/0.90

Radioactivity (μCi/g)
= 2{[(DPM-background DPM)/(2.22 X 10^6)]/sample weight}

Concentrations of TCB equivalents
= Radioactivity/specific activity of ^{14}C-TCB in feed

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Hamid, F. M., J. A. Moore, and H. B. Matthews. 1981. Comparative study of 3,4,3',4'-tetrachlorobiphenyl in male and female rats and female monkeys. J. Toxicol. Environ. Hlth. 7: 181-191.

Agrawal, A. K., H. A. Tilson, and S. C. Bondy. 1981. 3,4,3',4'-tetrachlorobiphenyl given to mice prenatally produces long-term decreases in striatal dopamine and receptor binding sites in the caudate nucleus. Toxicol. Lett. 7: 417-424.

Allen, J. R., D. A. Barsotti, L. K. Lambrecht, and J. P. Van Miller. 1979. Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. N. Y. Acad. Sci. 320: 419-425.

Anon. 1966. Report of a new chemical hazard. New Scientist. 32: 612.

Atlas, E., T. Bidleman, and C. S. Giam. 1986. Atmospheric transport of PCBs to the oceans. In: *PCBs and The Environment*. Volume I. pp. 79-100. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

Aulerich, R. J., S. J. Bursian, W. J. Breslin, B. A. Olson, and R. K. Ringer. 1985. Toxic2ological manifestations of 2,4,5,2',4',5'-, 2,3,6,2',3',6'-, and 3,4,5,3',4',5'-hexachlorobiphenyl and Aroclor 1254 in mink. J. Toxicol. Environ. Hlth. 15: 63-79.

Bandiera, S., S. Safe, and A. B. Okey. 1982. Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene-, or mixed-type inducers to cytosolic A*h* receptor. Chem. Biol. Interact. 39: 259-277.

Barsotti, D. A., R. J. Marlar, and J. R. Allen. 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Fd. Cosmet. Toxicol. 14: 99-103.

Bates, M. N., D. J. Hannah, S. J. Buckland, J. A. Taucher, and T. v. Maanen. 1994. Chlorinated organic contaminants in breast milk of New Zealand women. Environ. Hlth. Persp. Supple. 102(Supple 1): 211-217.

Bitman, J. and H. C. Cecil. 1970. Estrogenic activity of DDT analogs and polychlorinated biphenyls. J. Agr. Food Chem. 18(6): 1108-1112.

Bleavins, M. R., W. J. Breslin, R. J. Aulerich, and R. K. Ringer. 1984. Placental and mammary transfer of a polychlorinated biphenyl mixture (Aroclor 1254) in the European ferret (*Mustela putorius furo*). Environ. Toxicol. Chem. 3: 637-644.

Brandt, I., P. O. Darnerud, A. Bergman, and Y. Larsson. 1982. Metabolism of 2,4',5-trichlorobiphenyl: Enrichment of hydroxylated and methyl sulphone metabolites in the uterine luminal fluid of pregnant mice. Chem.-Biol. Interact. 40: 45-56.

Brezner, E., J. Terkel and A. S. Perry. 1984. The effects of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat-I. Comp. Biochem. Physiol. 77c(1): 65-70.

Burse, V. W., R. D. Kimbrough, E. C. Villaneuva, R. W. Jennings, R. E. Linder, and G. W. Sovocool. 1974. Polychlorinated biphenyls storage, distribution, excretion, and recovery: Liver morphology after prolonged dietary ingestion. Arch. Environ. Hlth. 29: 301-307.

Chen, P. H., K. T. Chang, and Y. D. Lu. 1981. Polychlorinated biphenyls and polychlorinated dibenzofurans in the toxic rice-bran oil that caused PCB poisoning in Taichung. Bull. Environ. Contam. Toxicol. 26: 489-495.

Chen, P. H. and S.-T. Hsu. 1989. PCB poisoning from toxic rice-bran oil in Taiwan. In: *PCBs and The Environment*. Volume III. pp. 27-38. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

Chen, S.-W., B. Magnus Francis, and P. J. Dziuk. 1993. Effects of concentration of mixed-function oxidase on concentration of estrogen, rate of egg lay, eggshell thickness, and plasma calcium in laying hens. J. Anim. Sci. 71: 2700-2707.

Chen, T. S. and K. P. Dubois. 1973. Studies of the enzyme inducing effect of polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 26:504-512.

Clarke, D. W., J. F. Brien, W. J. Racz, K. Nakatsu, and G. S. Marks. 1984. The disposition and the liver and thymus gland toxicity of 3,3',4,4'-tetrachlorobiphenyl in the female rat. Can. J. Physiol. Pharmacol. 62: 1253-1260.

Curley, A., V. W. Burse, and M. E. Grim. 1973. Polychlorinated biphenyls: Evidence of transplacental passage in the Sherman rat. Fd. Cosmet. Toxicol. 11: 471-476.

d'Argy, R., L. Denker, E. Klasson-Wehler, A. Bergman, P. O. Darnerud, and I. Brandt. 1987. 3,3',4,4'-Tetrachlorobiphenyl in pregnant mice: Embryotoxicity,

teratogenicity, and toxic effects on the cultured embryonic thymus. Pharmacol. Toxicol. 61: 53-57.

Darnerud, P. O., I. Brandt, E. Klasson-Wehler, A. Bergman, R. d'Argy, L. Dencker, and G. O. Sperber. 1986. 3,3',4,4'-Tetrachloro[14C]biphenyl in pregnant mice: Enrichment of phenol and methyl sulphone metabolites in late gestational fetuses. Xenobiotica 16(4): 295-306.

Dencker, L. and R. M. Pratt. 1981. Association between the presence of the Ah receptor in embryonic murine tissues and sensitivity to TCDD-induced cleft palate. Teratog. Carcinog. Mutag. 1: 399-406.

Duax, W. L. and J. F. Griffin. 1985. Structur-activity relationships of estrogenic chemicals. In: *Estrogens in The Environment II: Influence on Development*. PP. 15-23. Ed. J. A. McLachlan. Elsevier Science Publishing Co., Inc. New York, NY.

Ecobichon, D. J. and D. O. MacKenzie. 1974. The uterotropic activity of commercial and isomerically-pure chlorobiphenyl in the rat. Res. Commun. Chem. Pathol. Pharmacol. 9(1): 85-95.

Erickson, G. F., D. A. Magoffin, C. A. Dyer, and C. Hofeditz. 1985. The ovarian androgen producing cells: A review of structure/function relationships. Endocri. Rev. 6(3): 371-399.

Faith, R. E. and J. A. Moore. 1977. Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). J. Toxicol. Environ. Hlth. 3: 451-464.

Fishbein L. 1974. Toxicity of chlorinated biphenyls. Annu. Rev. Pharmacol. 14: 139-156.

Foster, P. M. D. 1989. Testicular structure and physiology: A toxicologist's view. In: *Toxicology of The Male and Female Reproductive Systems*. pp. 1-14. Ed. P. K. Working. Hemisphere Publishing Corporation, New York, NY.

Freeman, H. C., G. Sangalang, and B. Flemming. 1982. The sublethal effects of a polychlorinated biphenyl (Aroclor 1254) diet on the Atlantic cod (*Gadus morhua*). Sci. Total Environ. 24: 1-11.

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

Gage, J. C. and S. Holm. 1976. The influence of molecular structure of the retention and

excretion of polychlorinated biphenyls by the mouse. Toxicol. Appl. Pharmacol. 36: 555-560.

Gallenberg, L. A. and M. J. Vodicnik. 1987. Potential mechanism for redistribution of polychlorinated biphenyls during pregnancy and lactation. Xenobiotica 17(3): 299-310.

Garthoff, L. H., L. Friedman, T. M. Farber, K. K. Locke, T. J. Sobotka, S. Green, N. E. Hurley, E. L. Peters, G. E. Story, F. M. Moreland, C. H. Graham, J. E. Keys, M. J. Taylor, J. V. Scalera, J. E. Rothlein, E. M. Marks, F. E. Cerra, S. B. Rodi, E. M. Sporn. 1977. Biochemical and cytogenetic effects in rats caused by short-term ingestion of Aroclor 1254 or Firemaster BP6. J. Toxicol. Environ. Hlth. 3: 769-796.

Gellert, R. J. 1978. Uterotrophic activity of polychlorinated biphenyls (PCB) and induction of precocious reproductive aging in neonatally treated female rats. Environ. Res. 16: 123-130.

Gellert, R. J. and C. Wilson. 1979. Reproductive function in rats exposed prenatally to pesticides and polychlorinated biphenyls (PCB). Environ. Res. 18: 437-443.

George, F. W. and J. D. Wilson. 1988. Sex determination and differentiation. In: *The Physiology of Reproduction*. pp. 3-26. Eds. E. Knobil, J. D. Neill, L. L. Ewing, G. S. Greenwald, C. L. Markert, and D. W. Pfaff. Raven Press, New York, NY.

Golub, M. S., J. M. Donald, and J. A. Reyes. 1991. Reproductive toxicity of commercial PCB mixtures: LOAELs and NOAELs from animal studies. Environ. Hlth. Persp. 94: 245-253.

Gilula, N. B., W. Fawcett, and A. Aoki. 1976. The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testes. Dev. Biol. 50: 142-168.

Guo, Y. L., C. J. Lin, W. J. Yao, J. J. Ryan, and C. C. Hsu. 1994. Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). J. Toxicol. Environ. Hlth. 41: 83-93.

Haake-McMillan, J. M. and S. H. Safe. 1991. Neonatal exposure to Aroclor 1254: Effects on adult hepatic testosterone hydroxylase activities. Xenobiotica 21: 481-489.

Hadley, M. E. 1992. In: *Endocrinology*. 606 pp. Ed. M. E. Hadley. Pretice-Hall Inc., Englewood Cliffs, NJ.

Hansen, L. G. 1987. Food chain modification of the composition and toxicity of polychlorinated biphenyl (PCB) residues. Rev. Environ. Toxicol. 3: 149-212.

Harris, C. and W. S. Bradshaw. 1984. Alterations in liver ultrastructure and induction of UDP-glucuronyltransferase in the rat following prenatal exposure to 3,4,3',4'-tetrachlorobiphenyl. Arch. Environ. Contam. Toxicol. 13: 715-721.

Harris, G. W. 1964. Sex hormones, brain development and brain function. Endocri. 75:627-648.

Hemminki, K. and P. Vineis. 1985. Extrapolation of the evidence on teratogenicity of chemicals between humans and experimental animals: Chemicals other than drugs. Teratogen. Carcino. Mutagen. 5: 251-318.

Hileman, B. 1994. Environmental estrogens linked to reproductive abnormalities, cancer. C & EN. January. 19-23.

Hirayama, C. 1976. Clinical aspects of PCB poisoning. In: *PCB Poisoning and Pollution*. Ed. K. Higuchi. pp. 87-104. Academic Press, New York.

Hitomi, Y., M. Wakayama, H. Oda, and A. Yoshida. 1993. Liver-specific induction of NADPH-generating enzymes by polychlorinated biphenyls in rats. Biosci. Biotech. Biochem. 57(7): 1134-1136.

Jacobson, S. W., J. L. Jacobson, P. M. Schwartz, and G. G. Fein. 1983. Intrauterine exposure of human newborns to PCBs: Measures of exposure. In: *PCBs: Human and Environmental Hazards*. pp. 311-343. Eds. F. M. D'ltri and M. A. Kamrin. Butterworth Publisher, Woburn, MA.

Jonsson, H. T. Jr., J. E. Keil, R. G. Gaddy, C. B. Loadholt, G. R. Hennigar, and E. M. Walker, Jr. 1976. Prolonged ingestion of commercial DDT and PCB: Effects on progesterone levels and reproduction in the mature female rat. Arch. Environ. Contam. 3: 479-490.

Johansson, B. 1987. Lack of effects of polychlorinated biphenyls on testosterone synthesis in mice. Pharmacol. Toxicol. 61: 220-223.

Kashimoto, T. and H. Miyata. 1986. Differences between Yusho and other kinds of poisoning involving only PCBs. In: *PCBs and The Environment*. Vol. III. pp. 1-26. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

Kato, N. and A. Yoshida. 1980. Effect of dietary PCB on hepatic cholesterogenesis in rats. Nutr. Rep. Int. 21: 107-112.

Kholkute, S. D., J. Rodriguez, and W. R. Dukelow. 1994. Effects of polychlorinated biphenyls (PCBs) on *in vitro* fertilization in the mouse. Reprod. Toxicol. 8(1): 69-73.

Kihlstrom, J. E., C. Lundberg, J. Orberg, P. O. Danielsson, and J. Sydhoff. 1975. Sexual functions of mice neonatally exposed to DDT or PCB. Environ. Physiol. Biochem. 5: 54-57.

Kikuchi, M. and Y. Masuda. 1976. The pathology of Yusho. In: *PCB Poisoning and Pollution*. pp. 69-86. Ed. K. Higuchi. Kodansha Ltd. Tokyo.

Kilburn, K. H., R. H. Warsaw, and M. G. Shields. 1989. Neurobehavioral dysfunction in fireman exposed to polychlorinated biphenyls (PCBs): Possible improvement after detoxification. Arch. Environ. Hlth. 44(6): 345-350.

Klasson Wehler, E., A. Bergman, I. Brandt, P. O. Darnerud, and C. A. Wachtmeister. 1989. 3,3',4,4'-tetrachlorobiphenyl excretion and tissue retention of hydroxylated metabolites in the mouse. Drug Metab. Dispos. 17(4): 441-448.

Klasson Wehler, E., B. Brunstrom, U. Rannug, and A. Beggman. 1990. 3,3',4,4'-Tetrachlorobiphenyl: Metabolism by the chick embryo in ovo and toxicity of hydroxylated metabolites. Chem.-Biol. Interact. 73: 121-132.

Koga, N., M. Beppu, C. Ishida, and H. Yoshimura. 1989. Further studies on metabolism *in vivo* of 3,4,3',4'-tetrachlorobiphenyl in rats: Identification of minor metabolites in rat faeces. Xenobiotica. 19(11): 1307-1318.

Koga, N., M. Beppu, and H. Yoshimura. 1990. Metabolism *in vivo* of 3,4,5,3',4'-pentachlorobiphenyl and toxicological assessment of the metabolite in rats. J. Pharmacobio-Dyn. 13: 497-506.

Korach, K. S., P. Sarver, K. Chae, J. A. Mclachlan, and J. D. Mckinney. 1987. Estrogen receptor binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes. Mol. Pharmacol. 33: 120-126.

Kuo, J., E. Fox, and S. McDonald. 1992. In: *SigmaStat: User's Manual.* 13-60 pp. Jandel Scientific. San Rafael, CA.

La Rocca, P. T. and G. P. Carlson. 1979. The effect of polychlorinated biphenyls on adenosine triphosphatase activity. Toxicol. Appl. Pharmacol. 48: 185-192.

Leece, B., M. A. Denomme, R. Towners, S. M. Angela Li, and S. Safe. 1985. Polychlorinated biphenyls: Correlation between *in vivo* and *in vitro* quantitative structure-activity relationships (QSARs). J. Toxicol. Environ. Hlth. 16: 379-388.

Lincer, J. L. and D. B. Peakall. 1970. Metabolic effects of polychlorinated biphenyls in the American kestrel. Nature 228: 783-784.

Linzey, A. V. 1987. Effects of chronic polychlorinated biphenyls exposure on reproductive success of white-footed mice (*Peromyscus leucopus*). Arch. environ. Contam. Toxicol. 16: 455-460.

Linzey, A. V. 1988. Effects of chronic polychlorinated biphenyls on growth and reproduction of second generation white-footed mice (*Peromyscus leucopus*). Arch. Environ. Contam. Toxicol. 17: 39-45.

Lucier, G. W., O. S. McDaniel, C. M. Schiller, and H. B. Matthews. 1978. Structural requirements for the accumulation of chlorinated biphenyl metabolites in the fetal rat intestine. Drug Metab. Dispos. 6(5): 584-590.

Lucier, G. W. and O. S. McDaniel. 1979. Developmental toxicology of the halogenated aromatics: Effects on enzyme development. Ann. N. Y. Acad. Sci. 320: 449-457.

Lutz, R. J., R. L. Dedrick, H. B. Matthews, T. E. Eling, and M. W. Anderson. 1977. A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. Drug Metab. Dispos. 5: 386-396.

Lutz, R. J. and R. L. Dedrick. 1987. Physiologic pharmacokinetic modeling of polychlorinated biphenyls. In: *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*. pp. 111-131. Ed. S. Safe. Splinger-Verlag Press, Berlin.

Mably, T. A., R. W. Moore, and R. E. Peterson. 1992. *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1. Effects on androgenic status. Toxicol. Appl. Pharmacol. 114: 97-107.

MacLellan, K., A. Singh, I. Chu, and D. C. Villeneuve. 1994. Subchronic toxicity of 3,3',4,4'-tetrachlorobiphenyl in the rat liver: A electric microscope study. Histol. Histopath. 9:453-459.

Maliwal, B. P. and F. Guthrie. 1982. In vitro uptake and transfer of chlorinated hydrocarbons among human lipoproteins. J. Lipid Res. 23: 474-479.

Marks, T. A., G. L. Kimmel, and R. E. Staples. 1981. Influence of symmetrical polychlorinated biphenyl isomers on embryo and fetal development in mice. Toxicol. Appl. Pharmacol. 61: 269-276.

Marks, T. A. and R. E. Staples. 1980. Teratogenic evaluation of the symmetrical isomers of hexachlorobiphenyl (HCB) in the mouse. In: *Proc. 20th Annu. Meet.*

Teratol. Soc. p. 54A.

Matthews, H. B. and D. B. Tuey. 1980. The effect of chlorine position on the distribution and excretion of four hexachlorobiphenyl isomers. Toxicol. Appl. Pharmacol. 53: 377-388.

Matthews, H. B. and M. W. Anderson. 1975. Effects of chlorination on the distribution and excretion of polychlorinated biphenyls. Drug. Metab. Dispos. 3: 371-380.

Matthews, H. B. and R. L. Dedrick. 1984. Pharmacokinetics of PCBs. Ann. Rev. Pharmacol. Toxicol. 24: 85-103.

McConnell, E. E., J. A. Moore, J. K. Haseman, and M. W. Harris. 1978. The comparative Toxicity of chlorinated dibenzo-*p*-dioxin in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44: 335-356.

McConnell, E. E. and J. A. Moore. 1979. Toxicopathology characteristics of the halogenated aromatics. Ann. N. Y. Acad. Sci. 320: 138-150.

McConnell, E. E. 1985. Comparative toxicity of PCBs and related compounds in various species of animals. Environ. Hlth. Persp. 60: 29-33.

McConnell, E. E. 1989. Acute and chronic toxicity and carcinogenesis in animals. In: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. pp. 161-193. Eds. R. D. Kimbrough and A. A. Jensen. Elsevier, New York, NY.

McLachlan, M. S. 1993. Digestive tract absorption of polychlorinated dibenzo-*p*-dioxin, dibenzofurans, and biphenyls in a nursing infant. Toxicol. Appl. Pharmacol. 123: 68-72.

McNatty, K. P., D. M. Smith, A. Makris, R. Osathanondh, and K. J. Ryan. 1979. The microenvironment of the human antral follicle: Interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte *in vivo* and *in vitro*. J. Cli. Endocri. Meta. 49(6): 851-860.

Meistrich, M. L. 1986. Critical components of testicular function and sensitivity to disruption. Biol. Reprod. 34: 17-28.

Merson, M. H. and R. L. Kirkpatrick. 1975. Effects of dietary exposure to PCB on testicular characteristics, organ weights, and plasma corticoids of male white-footed mice. The Virginia Journal of Science 26: 58 (Abstract).

Mizutani, T., K. Hidaka, T. Ohe, and M. Matsumoto. 1977. A comparative study on accumulation and elimination of tetrachlorobiphenyl isomers in mice. Bull. Environ. Contam. Toxicol. 18(4): 452-461.

Morrissey, R. E. and B. A. Schwetz. 1989. Reproductive and developmental toxicity in animals. In: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. pp. 195-225. Eds. R. D. Kimbrough and A. A. Jensen. Elsevier, New York.

Neal, R. A. 1985. Mechanisms of the biological effects of PCBs, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in experimental animals. Environ. Hlth. Persp. 60: 41-46.

Nebert, D. W. 1989. The Ah locus: Genetic differences in toxicity, cancer, mutation, and birth defects. Critical Rev. Toxicol. 20(3): 137-152.

Orberg, J. and J. E. Kihlstrom. 1973. Effects of long-term feeding of polychlorinated biphenyls (PCB, Clophen A 60) on the length of the oestrous cycle and on the frequency of implanted ova in the mouse. Environ. Res. 6: 176-179.

Orberg, J. 1978a. Effects of pure chlorobiphenyls (2,4',5-trichlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl) on the reproductive capacity in female mice. Acta. Pharmacol. et. Toxicol. 42: 323-327.

Orberg, J. 1978b. Effects of pure chlorobiphenyls (2,4',5-trichlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl) on the post-natal growth. Acta. Pharmacol. et. Toxicol. 42: 275-279.

Overmann, S. R., J. Kostas, L. R. Wilson, W. Shain, and B. Bush. 1987. Neurobehavioral and somatic effects of perinatal PCB exposure in rats. Environ. Res. 44: 56-70.

Pantaleoni, G., D. Fanini, A. M. Sponta, G. Palumbo, R. Giorgi, and P. M. Adams. 1988. Effects of maternal exposure to polychlorobiphenyls (PCBs) on F1 generation behavior in the rat. Fundam. Appl. Toxicol. 11: 440-449.

Parkinson, A. and S. Safe. 1981. Aryl hydrocarbon hydroxylase induction and its relationship to the toxicity of halogenated aryl hydrocarbons. Toxicol. Environ. Chem. Rev. 4: 1-45.

Parkinson, A., S. H. Safe, L. W. Robertson, P. E. Thomas, D. E. Ryan, L. M. Reik, and W. Levin. 1983. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydroxylase in liver microsomes from polychlorinated or polychlorinated biphenyl-treated rats. J. Biol. Chem. 258(9): 5967-5976.

Parkinson, A. and S. Safe. 1987. Mammalian biologic and toxic effects of PCBs. In: *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*. pp. 49-75. Ed. S. Safe. Splinger-Verlag Press, Berlin.

Peltola, V., E. Mantyla, I. Huhtaniemi, and M. Ahotupa. 1994. Lipid peroxidation and antioxidant enzyme activities in the rat testis after cigarette smoke inhalation or administration of polychlorinated biphenyls or polychlorinated naphthalenes. J. Androl. 15(4): 353-361.

Peters, H. 1979. Some aspects of early follicular development. In: *Ovarian Follicular Development and Function*. pp. 1-15. Eds. A. R. Midgley, Jr. and W. A. Sadler. Plenum, New York, NY.

Peterson, R. E., H. M. Theobald, and G. L. Kimmel. 1993. Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons. Crit. Rev. Toxicol. 23(3): 283-335.

Phillips, D. J. H. 1986. Use of organism to quantify PCBs in marine and estuarine environments. In: *PCBs and The Environment*. Vol. II. pp. 127-181. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

Platonow, N. S. and H. S. Funnell. 1971. Anti-androgenic-like effects of polychlorinated biphenyls in cockerels. Vet. Rec. 89:109-110.

Poland, A. and J. Knutson. 1982. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Ann. Rev. Pharmacol. Toxicol. 22: 517-554.

Rands, P. L., R. D. White, M. W. Carter, S. D. Allen, and W. S. Bradshaw. 1982. Indicators of developmental toxicity following prenatal administration of hormonally active compounds in the rat. I. Gestational length. Teratol. 25: 37-43.

Richards, J. S. 1980. Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. 60(1): 51-89.

Richardson B. J. and J. S. Waid. 1982. Polychlorinated Biphenyls (PCBs): An Australian viewpoint on a global problem. Search 13 (1-2): 17-25.

Ringer, R. K., R. J. Aulerich, and M. Zabik. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Proceedings of the 164th National Meeting of the American Chemical Society. P. WATR41.

Risebrough, R. and V. Brodine. 1970. More letters in the wind. Environ. 12(1): 16-27.

Rogan, W. J. 1982. PCBs and cola-colored babies: Japan, 1968, and Taiwan, 1979. Teratol. 26: 259-261.

Rogan, W. J., B. C. Gladen, K.-L. Hung, S.-L. Koong, L.-Y. Shih, J. S. Taylor, Y.-C. Wu, D. Yang, N. B. Ragan, and C.-C. Hsu. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241: 334-336.

Ronnback, C. 1991. Effects of 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovaries of foetal mice. Pharmacol. Toxicol. 69:340-345.

Ronnback, C. and D. G. de Rooij. 1994. Effects of 3,3',4,4'-tetrachlorobiphenyl on foetal germ cells in two mouse strains after repeated treatment of the dams during and after pregnancy. Pharmacol. Toxicol. 74: 287-293.

Ryan, K. J. 1988. Endocrine function of the ovary. In: *Endocrinology: People and Ideas*. pp. 201-213. Ed. S. M. McCann. Waverly Press, Inc., Baltimore, MD.

Safe, S. 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology, and mechanism of action. CRC Crit. Rev. Toxicol. 13(4): 319-395.

Safe, S. 1989. Polyhalogenated aromatics: Uptake, disposition and metabolism. In: *Halogenated Piphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. pp. 131-159. Eds. R. D. Kimbrough and A. A. Jensen. Elsevier, New York, NY.

Safe, S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit. Rev. Toxicol. 21: 51-88.

Safe, S., L. Safe, and M. Mullin. 1987. Polychlorinated biphenyls: Environmental occurrence and analysis. In: *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*. pp. 1-13. Eds. S. Safe and O. Hutzinger. Springer-Verlag Press, Berlin.

Safe, S. H. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24(2): 87-149.

Sager, D. B. 1983. Effect of postnatal exposure to polychlorinated biphenyl on adult male reproductive function. Environ. Res. 31: 76-94.

Sager, D. B., W. Shih-Schroeder, and D. Girard. 1987. Effects of early postnatal exposure to polychlorinated biphenyls (PCBs) on fertility in male rats. Bull. Environ. Contam. Toxicol. 38: 946-953.

Sager D., D. Girard, and D. Nelson. 1991. Early postnatal exposure to PCBs: Sperm function in rats. Environ. Toxicol. Chem. 10:737-746.

Sanders, O. T. and R. L. Kirkpatrick. 1975. Effects of a polychlorinated biphenyl (PCB) on sleeping times, plasma corticosteroids, and testicular activity of white-footed mice. Environ. Physiol. Biochem. 5: 308-313.

Sanders, O. T., R. L. Kirkpatrick, and P. E. Scanlon. 1977. Polychlorinated biphenyls and nutritional restriction: Their effects and interactions on endocrine and reproductive characteristics of male white mice. Toxicol. Appl. Pharmacol. 40: 91-98.

Sanders, O. T., R. L. Zepp, and R. L. Kirkpatrick. 1974. Effects of PCB ingestion on sleeping times, organ weights, food consumption, serum corticosterone and survival of albino mice. Bull. Environ. Contam. Toxicol. 12 (4): 394-399.

Sawhney, B. L. 1986. Chemistry and properties of PCBs in relation to environmental effects. In: *PCBs and The Environment*. Vol. I. pp. 47-64. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

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

Schecter, A., J. Stanley, K. Boggess, Y. Masuda, J. Mes, M. Wolff, P. Furst, C. Furst, K. Wilson-Yang, and B. Chisholm. 1994. Polychlorinated biphenyl levels in the tissues of exposed and nonexposed humans. Environ. Hlth. Persp. Supple. 102(Supple 1): 149-158.

Schlebusch, H., U. Wagner, H. van der Ven, S. Al-Hasani, K. Diedrich, and D. Krebs. 1989. Polychlorinated biphenyls: The occurrence of the main congeners in follicular and sperm fluids. J. Clin. Chem. Clin. Biochem. 27: 663-667.

Sever, L. E. and N. A. Hessol. 1985. Toxic effects of occupational and environmental chemicals on the testes. In: *Endocrine Toxicology*. pp. 211-248. Eds. J. A. Thomas, K. S. Korach, and J. A. McLachlan. Raven Press, New York, NY.

Shaw, G. R. and D. W. Connell. 1990. Factors controlling bioaccumulation of PCBs. In: *PCBs and The Environment*. Vol. I. pp. 135-141. Ed. J. S. Waid. CRC Press, Boca Raton, FL.

Simmons, D. L., D. M. Valentine, and W. S. Bradshaw. 1984. Different patterns of developmental toxicity in the rat following prenatal administration of structurally adverse chemicals. J. Toxicol. Environ. Hlth. 14: 121-136.

Sipes, I. G. and A. J. Gandolfi. 1991. Biotransformation of toxicants In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. pp. 88-126. Eds. M. O. Amdur, J. Doull, and C. D. Klaassen. Pergamon Press, Elmsford, NY.

Sipes, I. G. and R. G. Schnellmann. 1987. Biotransformation of PCBs: Metabolic pathways and metabolisms. In: *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*. pp. 97-110. Ed. S. Safe. Splinger-Verlag Press, Berlin.

Smialowicz, R. J., J. E. Andrews, M. M. Riddle, R. R. Rogers, R. W. Luebke, and C. B. Copeland. 1989. Evaluation of the immunotoxicity of low level PCB exposure in the rat. Toxicol. 56: 197-211.

Spindler-Vomachka, M. and M. J. Vodicnik. 1984. Distribution of 2,4,5,2',4',5'-hexachlorobiphenyl among lipoproteins during pregnancy and lactation in the rat. J. Pharmacol. Exp. Ther. 230(2): 263-268.

Stone, R. 1994. Environmental estrogens stir debate. Science. 265: 308-310.

Takagi, Y., S. Aburada, K. Hashimoto, and T. Kitaura. 1986. Transfer and distribution of accumulation (^{14}C) polychlorinated biphenyls from maternal to fetal and suckling rats. Arch. Environ. Contam. Toxicol. 15: 709-715.

Takagi, Y., T. Otake, M. Kataoka, Y. Murata, S. Aburada, S. Akasaka, K. Hashimoto, H. Uda, and T. Kitaura. 1976. Studies on the transfer and distribution of [^{14}C] polychlorinated biphenyls from maternal to fetal and suckling rats. Toxicol. Appl. Pharmacol. 38: 549-558.

Tanabe, S., Y. Nakagawa, and R. Tatsukawa. 1981. Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. Agric. Biol. Chem. 45: 717-726.

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

Thomas, J. A. 1991. Toxic responses of the reproductive system. In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. pp. 484-520. Eds. M. O. Amdur, J. Doull, and C. D. Klaassen. Pergamon Press. New York, NY.

Tilson, H. A., G. J. Davis, J. A. McLachlan, and G. W. Lucier. 1979. The effects of

polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. Environ. Res. 18: 466-474.

Topham, J. C. 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutation Res. 74: 379-387.

Truelove, J. F., J. R. Tanner, I. A. Langlois, R. A. Stapley, D. L. Arnold, and J. C. Mes. 1990. Effect of polychlorinated biphenyls on several endocrine reproductive parameters in the female rhesus monkey. Arch. Environ. Contam. Toxicol. 19: 939-943.

ven den Berg, K. J., C. Zurcher, A. Brouwer, and D. W. van Bekkum. 1988. Chronic toxicity of 3,3',4,4'-tetrachlorobiphenyl in the marmoset monkey (*Callithrix jacchus*). Toxicol. 48: 209-224.

Vergouwen, R. P. F. A., R. Huiskamp, R. J. Bas, H. L. Roepers-Gajadien, J. A. G. Davis, and D. G. de Rooij. 1993. Postnatal development of testicular cell populations in mice. J. Reprod. Fert. 99: 479-485.

Villeneuve, D. C. and D. L. Grant. 1971. Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bull. Environ. Contam. Toxicol. 6(2): 120-128.

Vincent, D. R., W. S. Bradshaw, G. M. Booth, R. E. Seegmiller, and S. D. Allen. 1992. Effect of PCB and DES on rat monoamine oxidase, acetylcholinesterase, testosterone and estradiol ontogeny. Bull. Environ. Contam. Toxicol. 48: 884-893.

Vodicnik, M. J. and J. J. Lech. 1980. The transfer of 2,4,5,2',4',5'-hexachlorobiphenyl to fetuses and nursing offspring. Toxicol. Appl. Pharmacol. 54: 293-300.

Vodicnik, M. J. 1986. The effect of pregnancy and lactation on the disposition of [2,4,2',4'-^{14}C]tetrachlorobiphenyl in the mouse. Fundam. Appl. Toxicol. 6: 53-61.

Voet, D. and J. G. Voet. 1990. In: *Biochemistry*. 1223 pp. Eds. D. Voet and J. G. Voet. John. Wiley and Sons, New York, NY.

Vos, J. G. and R. B. Beems. 1971. Dermal Toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. Toxicol. Appl. Pharmacol. 19: 617-633.

Ward, J. M. 1985. Proliferative lesions of the glandular stomach and liver in F334 rats fed diets containing Aroclor 1254. Environ. Hlth. Persp. 60: 89-95.

Yoshimura, H. and H. Yamamoto. 1974. Metabolic studies on polychlorinated biphenyls. IV. Biotransformation of 3,4,3',4'-tetrachlorobiphenyl, one of the major components of Kanechlor-400. Fukuoka Acta Med. 65(1): 5-11.

Yoshimura, H., S. Yoshihara, N. Ozawa, and M. Miki. 1979. Possible correlation between induction modes of hepatic enzymes by PCBs and their toxicity in rats. Ann. N. Y. Acad. Sci. 320: 179-192.

Yoshimura, H., Y. Yonemoto, H. Yamada, N. Koga, K. Oguri, and S. Saeki. 1987. Metabolism *in vivo* of 3,3',4,4'-tetrachlorobiphenyl and toxicological assessment of the metabolites in rats. Xenobiotica. 17(8): 897-910.