THE ENVIRONMENTAL CONTAMINANTS, PCBs IMPACT ON ANDROGEN RECEPTOR ACTION AND PROSTATE GROWTH AND DEVELOPMENT

by

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We accept this thesis as conforming
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ABSTRACT

Polychlorinated biphenyls (PCBs) are synthetic chemicals that were used in industrial lubricants and household goods until they were banned in open systems in North America in 1977, after millions of tons were manufactured. PCBs are currently widespread major environmental contaminants that persist in the environment and due to their lipophilic nature, bioaccumulate exponentially up the food chain. All humans, particularly in industrialized countries, carry a burden of PCBs primarily in their adipose tissue. Studies in laboratory animals, wildlife, and humans exposed to PCB spills suggest that PCBs interfere with normal steroid hormone action. Therefore, PCBs and other persistent organochlorines, pose a risk to human health as endocrine disrupting compounds that may target hormone sensitive organs such as the prostate which require steroid hormones for appropriate growth and differentiation. *In utero* exposure to compounds that affect steroid hormone action can be the most detrimental to development.

In this study the influence of PCBs on the androgen axis *in vitro* and *in vivo* were examined. The effects of PCBs on androgen and glucocorticoid regulated reporter gene expression in a prostate cell line, and the ability of PCBs to influence binding of endogenous hormones to the androgen receptor (AR) were tested. Aroclor 1254 acted as a weak androgen receptor agonist, and Aroclor 1254, Aroclor 1242, Aroclor 1260, PCB 42, and PCB 31 were antagonistic to androgen activity at high concentrations relative to natural ligand concentrations. PCB 42 was an additive glucocorticoid receptor (GR) agonist and increased reporter activity to a level 150% higher at 10000 nM than at 0 nM PCB 42. Aroclor 1254 and PCB 42 significantly reduced the levels of DHT binding to

the androgen receptor by 25% and 50% respectively, at 1000 nM. Both these compounds reduced DHT levels by 90 to 95% at 10000 nM.

The treatment of mice transgenic for a prostate specific probasin promoter driven a CAT reporter gene, with Aroclor 1254 resulted in changes in prostate growth and development. In a single dose level study, mice were treated *in utero* and until four weeks (prepubertal) and eight weeks (post pubertal) of age with 10 mg/kg/day of Aroclor 1254. Additionally, in a dose response study, mice were treated *in utero* and until eight weeks of age with 10, 20, and 40 mg/kg/day. Prostate, testis, epididymis, heart, kidney, and liver weights were measured for comparison between groups. Results indicated that Aroclor 1254 has the capacity to reduce prostate weight and increase liver weight (both normalized for body weight) in a dose dependent manner. Finally, prostatic CAT activity was measured. This PCB mixture significantly reduced CAT activity in eight week old treatment mice in the single dose level study. Histology of prostate and liver were also examined in the dose response group. Histological alterations included dose related changes in liver vacuolization, as well as lymphocytic infiltrations and an increase in the presence of predominantly dilated acini in the prostate in a dose dependent manner. These findings demonstrate that PCBs can influence steroid hormone action through the androgen axis *in vitro*, and interfere with androgen-regulated gene expression *in vivo*.

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LIST OF ABBREVIATIONS

ABP =	Androgen Binding Protein
AhR =	Aryl Hydrocarbon Receptor
ANOVA =	Analysis of Variance
APEOs =	Alkylphenol Polyethoxylates
AR =	Androgen Receptor
CAT =	Chloramphenicol Acetyl Transferase
CYP =	Cytochrome P450
DBD =	DNA Binding Domain
DES =	Diethylstilbesterol
DEX =	Dexamethasone
DHT =	Dihydrotestosterone
DMEM =	Dulbecco's Modified Eagle's Medium
EDTA =	Ethylenediaminetetraacetic Acid
EGTA =	Ethyleneglycol-bis[ß-aminoethylether]-N,N,N,N',N'-tetraacetic Acid
ER =	Estrogen Receptor
GR =	Glucocorticoid Receptor
HEPES =	N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid
LBD =	Ligand Binding Domain
LD_{50} =	Dose which is Lethal to 50% of the Test Animals
LOAEL =	Lowest Observed Adverse Effect Level
MFOs =	Mixed Function Oxidases
MR =	Mineralocorticoid Receptor
NBF =	Neutral Buffered Formalin
NOAEL =	No Observed Adverse Effect Level
PCB =	Polychlorinated Biphenyl
PCDF =	Polychlorinated Dibenzofuran
PR =	Progesterone Receptor
SPBs =	Serum Binding Proteins
S-FBS =	Stripped Fetal Bovine Serum
SEM =	Standard Error of the Mean
T3 =	Triiodothyronine
T4 =	Thyroxine
TCDD =	2,3,4,8-Tetrachlorodibenzo-P-Dioxin
TEF =	TCDD Equivalency Factor
TR =	Thyroid Hormone Receptor

CHAPTER 1.

INTRODUCTION

1.1 Endocrine Disruptors

1.1.1 Discovery

The concept of endocrine disruptors has come to light over the past 60 years, as scientists have begun to piece together evidence of alarming changes in wildlife populations. The disappearance of mammal and bird populations in Europe and North America, and the high incidences of behavioral abnormalities, and reproductive and developmental defects were initially inexplicable. These anomalies were observed in invertebrates, fish, reptiles, birds, and mammals (including humans). The possibility that this phenomenon was due to environmental contaminants was considered (*1*). Abnormalities in specific groups in locations with high levels of pesticides and other organochlorine compounds suggested the link between environmental contaminants and the observed alterations in wildlife species (*2-6*). A decrease in sperm counts and sperm quality during the 20^{th} century was reported in men from Asia, South America, North America, and Europe (*7*). The rate of such changes could not be explained simply by genetic factors, and again, environmental contaminants became suspect (*8*).

Endocrine disrupting compounds mimic, inhibit, or alter the ability of natural hormones to act in the regulation of endocrine systems (*9*). More specifically, they are exogenous agents that interfere with the synthesis, storage, release, transport, metabolism, binding, action or elimination of natural hormones (*1*). Endogenous hormones regulate homeostasis and development, and thus alteration of these processes

causes disruption in critical aspects of cellular, organ, and organismal function. There is a wide range of effects on steroid hormone systems due to exposure to endocrine disruptors which depends on the nature of the compound such as toxicity, and persistence, as well as the amount, time of life that exposure occurs, and duration of exposure (*10*).

1.1.2 Target Sites

Endocrine modulating compounds can affect a number of systems in the body. The more common sites of disruption include the immune system, thyroid function, and neurodevelopment and behaviour (*10*). These chemicals have also been linked to hormone-related cancers such as those of the breast, testis, and prostate, and can be harmful to the reproductive system (*9*).

The threat of reproductive effects such as alterations in hormone balance and production (*4*), developmental abnormalities of the reproductive tract (*11*), and a global decrease in human sperm counts (*8*) implicates endocrine disruption as a contributing factor to the compromised reproductive fitness of many species, including humans.

1.1.3 Mechanisms of Action of Endocrine Disruptors

Different mechanisms of action of environmental contaminants have been determined as pathways that lead to impaired steroid hormonal control. Normal steroid hormone function begins with a small molecule synthesized from blood cholesterol and absorbed into the appropriate gland (*12*). These molecules generally act at the level of transcription initiation and affect mRNA synthesis, which results in a modification in protein synthesis. Once a steroid hormone enters the cell, it forms a high affinity complex with the cognate steroid hormone receptor by binding to the ligand binding

domain. The complex enters the cell nucleus and binds as a dimer to DNA via an interaction between a discrete domain in the protein with a specific response element sequence in the regulatory region of the target genes. Recruitment of coregulatory proteins to the DNA-bound dimer enables the hormone to modulate gene transcription and ultimately protein levels (*12*). The synthesized proteins mediate the effects of steroid hormones on target tissues including metabolic activation of cellular growth, and activation of cellular differentiation.

In the case of the steroid hormone testosterone (Figure 1), this hormone is synthesized mainly in the testes, through a reaction involving conversion from androstenedione by the action of 17ß-hydroxysteroid oxidoreductase. Most of the biosynthesized testosterone from the testis goes into peripheral circulation and is bound to serum binding proteins (SBPs), although 2% of the total in the plasma is free testosterone and remains unbound. A fraction of the testosterone diffuses from the Leydig cells into the seminiferous tubules, Sertoli cells and germ cells for the maintenance of spermatogenesis and to the genital tract, including the epididymis. One of the mechanisms by which testosterone reaches the epididymis, is through transport by androgen binding protein (ABP). Testosterone also contributes to the biosynthesis of ABP, and is involved in the activation of spermatozoa (*12*).

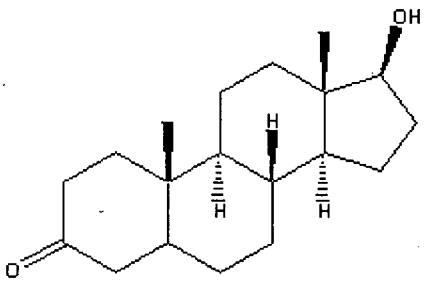
Testosterone is converted to dihydrotestosterone (DHT) (Figure 1) by 5α-reductase. DHT exerts it action by binding to the ligand binding domain of the AR, enters the nucleus and binds as a dimer through the DNA binding domain to nuclear DNA. Coregulatory proteins are recruited and androgen regulated responses in the cell are initiated through gene transcription and protein production.

T and other steroid hormones are made by a series of biosynthetic enzymes and are metabolized by enzymes known as mixed function oxidases (MFOs) which are part of the cytochrome P450 (CYP) superfamily of enzymes involved in the synthesis and catabolism of steroid hormones and in the detoxification of xenobiotics (*13*). These processes enable the synthesis of steroid hormones from cholesterol, and transform these hormones from one type to another. For example, aromatase transforms androgens to estrogens (*12*).

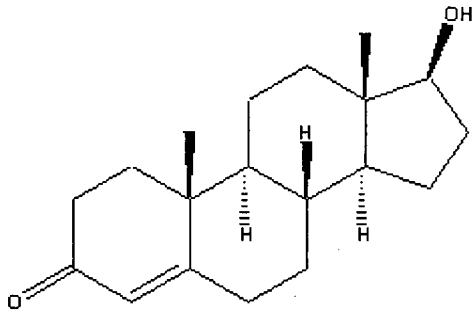
The catabolism of steroid hormones involves reductions and hydroxylations to conjugate the steroid with an acid to allow for elimination, which is mostly through the urine. Many of these reactions take place in the liver, particularly in the case of androgens.

An endocrine disruptor can alter the synthesis of steroid hormones (*14*) or alter the transport and clearance of hormones by binding to steroid binding proteins in the serum. This prevents endogenous steroid hormones from binding to these carrier proteins, resulting in an increased level of steroid hormones within the cell (*15*). Environmental hormones are also known to alter steroid hormone receptor activation indirectly by downregulating the receptor by decreasing sensitivity of the receptor to the ligand (*9*).

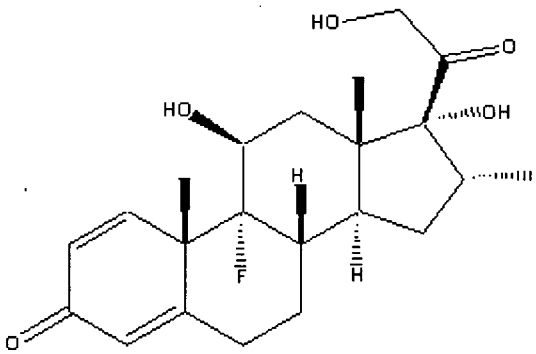
Finally, the most common known mechanism of endocrine disruption is through direct hormone receptor binding by the contaminant and its metabolites. Steroid hormone receptors including the AR and estrogen receptor (ER) are considered to have a significant amount of latitude in binding specificity (*16, 17*).



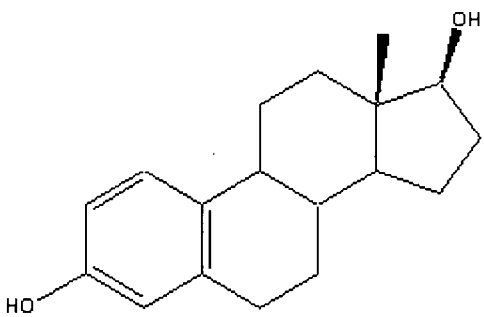
Dihydrotestosterone (DHT)



Testosterone



Dexamethasone (DEX)



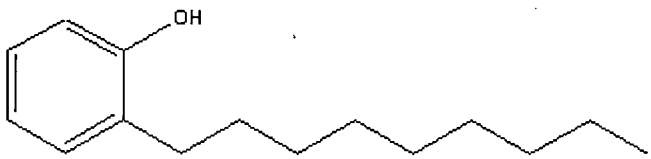
Estradiol

Figure 1. Chemical structure of some steroid hormones.

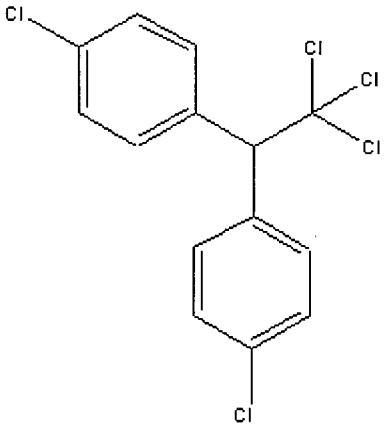
By mimicking the natural ligand or inhibiting the natural ligand from binding to the receptor, a compound can act as a steroid hormone agonist or antagonist, respectively. This has been shown through *in vitro* hormone binding and transcriptional assays that demonstrated the ability of many environmental contaminants to interact at the molecular level with one or more steroid hormone receptors (*17-20*).

1.1.4 Diethylstilbesterol

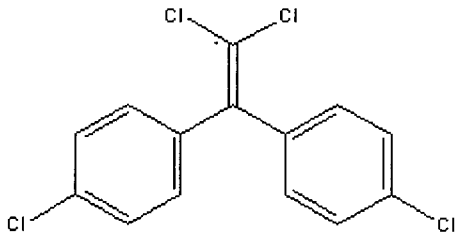
One of the first compounds found to interfere with normal hormone action was diethylstilbesterol (DES) (Figure 2). This drug is a synthetic estrogen discovered in 1938 and administered to pregnant women to prevent miscarriages and premature births. It was also used to suppress lactation after childbirth and to reduce symptoms of menopause. In agriculture, DES was used to increase the rate of weight gain in livestock. In the 1970s the teratogenic nature of DES was discovered. This highly estrogenic non-steroidal chemical causes reproductive abnormalities such as vaginal adenocarcinoma in females (*21*) and hypospadias and other genital tract abnormalities in males (*22, 23*) exposed *in utero*. DES has also been associated with a higher risk of breast cancer in women who took this drug during pregnancy to prevent miscarriages (*23*). Some of these abnormalities include effects on the prostate. Animal studies have shown that pregnant female mice fed 200 ug/kg/day have male offspring with reduced prostate weights (*24, 25*). A reduction in prostate weight was also observed in male offspring of female rats whose drinking water contained 100 µg/l of DES (*26*). By contrast, lower doses of DES ranging from 0.02 to 2 ng/kg/day to female mice during gestation caused an increase in prostate weight in the male offspring in adulthood (*24, 25*).



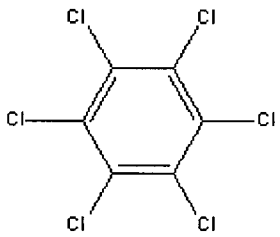
Nonylphenol



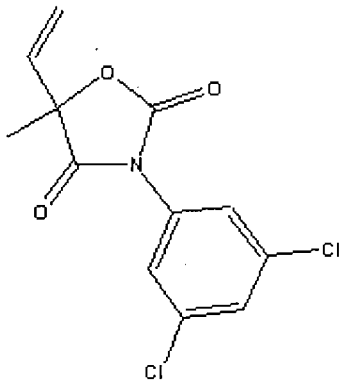
DDT



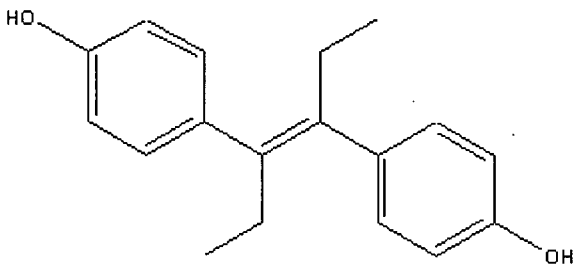
p,p'-DDE



Hexachlorobenzene



Vinclozolin



Diethylstilbesterol (DES)

Figure 2. Chemical structures of some known endocrine disruptors in the environment.

In utero exposure to DES has been linked to compromised immune function in animal models (*27, 28*) and in humans (*7*). The high potency of DES can be partly explained because it has a low binding affinity for serum hormone binding proteins and is therefore more bioavailable than endogenous estrogens. This characteristic suggests that *in vivo*, DES can cross the placental barrier and accumulate inside target cells at a higher rate than estradiol (*10*).

The unfortunate finding that this drug which millions of people were exposed to is carcinogenic and teratogenic was an important finding regarding the harmful effects of synthetic agents that act as hormones, particularly *in utero*.

1.1.5 Endocrine Disruptors in the Environment

In addition to DES, the existence of other compounds that possess endocrine disrupting activity has been discovered. Most of these chemicals are organohalide environmental contaminants such as pesticides, fungicides, herbicides, alkylphenols, and polychlorinated biphenyls (PCBs) (*29*). The Canadian Environmental Protection Agency and the United States Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC) are examining chemicals in the environment for endocrine disrupting potential. There are currently 87 000 chemicals in commerce which require evaluation for all potential endocrine disrupting capacities (*30*). Over 120 chemicals that are released into the environment have been found to have endocrine disrupting activities (*10*).

The pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) (Figure 2) and its metabolites are quite lipophilic, poorly metabolized, and bioaccumulate in the food chain (*11*). DDT was banned in the U.S. in 1972, although it is still in use in developing

countries and continues to accumulate in the global environment through air currents (*11*). This compound is therefore found ubiquitously in all individuals at varying levels. Reduced breeding in fish-eating birds has been attributed to the environmental toxicity of DDT (*7*).

This compound binds to the ER, activating estrogen regulated gene transcription and increasing estrogen metabolism (*10, 31*). The DDT metabolite, p,p'-DDE (Figure 2) binds to the AR and is antagonistic, therefore causing inhibition of androgen regulated sexual differentiation in rodents and reptiles at environmentally relevant levels (*19*).

The dicarboxamide fungicide vinclozolin (3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-oxazolidine-2,4-dione) (Figure 2) is used to control fungal growth on fruits, vegetables, ornamental plants, and turfgrass (*32*). This compound has also been shown to have endocrine disrupting capabilities. Vinclozolin alters sexual differentiation in male rats treated *in utero* and modifies their development in an antiandrogenic manner (*33*). Metabolites of vinclozolin (M1 and M2) compete with DHT for binding to the AR and inhibit DHT-induced transcriptional activity *in vitro* (*18*).

The fungicide hexachlorobenzene (Figure 2) was originally introduced in 1945 for agricultural use as a seed protectant treatment, particularly to control bunt growth on wheat. This chemical interferes with mechanisms that regulate ovarian steroidogenesis (*34*), alters menstrual cycle characteristics (*35*), and decreases progesterone serum levels (*36*) in mammals.

Alkylphenol polyethoxylates (APEOs) are nonionic surfactants, which have been produced since the 1940s. These compounds are widely used in detergents, herbicides, pesticides, and paints. APEOs and their metabolites, such as nonylphenol (Figure 2) are

released through sewage treatment and are leached from plastics, particularly when heated. APEOs have been known to displace estrogen from the ER since 1978 (*20*). The observation that polystyrene tubes can release nonylphenols, which stimulate the estrogen-dependent growth of MCF-7 breast adenocarninomca cells (*15*), was a powerful endocrine effect that brought attention back to this group of endocrine disruptors.

The ubiquitous environmental contaminants PCBs (Figure 3), the focus of this study, are also considered to have endocrine disrupting potential, in addition to a number of other health effects (*37*). The magnitude of the effects of endocrine disruptors on steroid hormone systems is vast and certainly not yet fully elucidated. By determining the endocrine effects of environmental contaminants such as PCBs, we may begin to understand and address the rapidly changing state of human and ecological reproductive health.

1.2 Polychlorinated Biphenyls (PCBs)

1.2.1 Industrial Use of PCBs

Polychlorinated biphenyls are persistent, toxic environmental pollutants which have bioaccumulated in the environment. These compounds were produced commercially as mixtures of up to 209 isomers or congeners. PCBs have been manufactured in the USA since 1929 and had common commercial use, mainly as coolants and lubricators in electrical equipment by 1930 (*38*). PCBs also became common components of general use products such as solvent extenders, flame retardants, organic diluents, inks, dyes, paints, and adhesives, and were found in carbonless copy paper, newsprint, and caulking compounds (*39*).

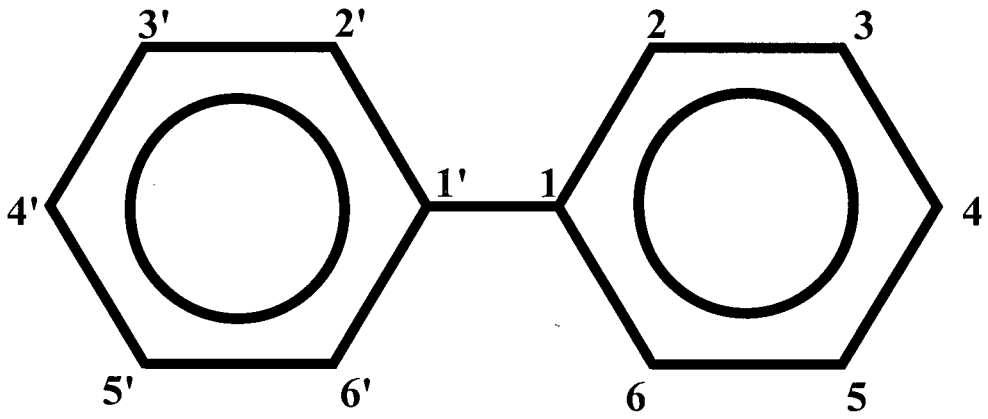


Figure 3. The general chemical strucutre of chlorinated bipheynls (ATSDR, 1998).

Although PCB manufacture was generally banned in the United States in the late 1970s, millions of tons were released into the environment between 1929 and 1977 and are still detected in all levels of the food chain in a biomagnified manner (*37*), as PCBs become further concentrated in animals with each successive step up the food chain (*40*). Slow accumulation of PCBs in adipose tissues over time leads to tissue concentrations far above acutely toxic intake levels that would be detected in the blood, immediately after exposure. However, through the food chain, a predator can ingest a fairly high dose of PCBs from its prey. This process continues up to the top predators as PCB biomagnification takes place (*41*). For example, in the Lake Michigan food web PCBs increase 12.9 times from plankton to fish (*42*).

1.2.2 PCB Exposure in the General Population and Groups with High Levels of Exposure

PCBs are highly stable compounds, which are fat soluble, bioaccumulative, and ubiquitously present in all humans. The estimated daily exposure in the United States of America from 1982 to 1984 was approximately 40 ng/day (*43*). In this study, 85% of the PCBs that adult men acquired from their diet was from meat products (*43*). The offspring of women exposed to PCBs also experience PCB exposure *in utero* and during lactation. Even though PCBs are detected in human tissues and breast milk at levels which are considered low regarding most toxicological endpoints, these concentrations have been reported to be harmful to reproductive, developmental, and endocrine processes (*44, 45*). The average PCB concentration in whole human breast milk ranges from 10 to 180 ng/ml, which leads to a range of 1.5 to 27 μg/kg/day of PCBs that breast fed infants may consume (*46*). Infants and young children consume more food per body weight than

adults, and may have a greater relative exposure to PCBs than adults which may contribute to the higher susceptibility of infants and young children to PCBs (*47*). PCBs have been reported to cross the placenta in a study which correlated the concentration of PCBs in maternal serum (4.7 ng/ml) to that in cord serum (2 ng/ml) (*48*). Due to their lipophilic nature, PCBs are stored in adipose tissues. Therefore, it should be noted that while many studies report PCB levels in serum, this value is likely well below the levels stored in tissues such as fat.

Individuals exposed to PCBs by occupational exposure or ingestion of highly contaminated foods can have much higher body burdens of PCBs. Capacitor plant workers in the United States have reported serum PCB levels ranging from 1 up to 1700 ng/ml (*49*). People who eat a diet rich in Great Lakes fish have serum PCB levels ranging from 7 to 366 ng/ml, depending on the amount of fish they consumed (*49*). Individuals exposed to PCBs by eating PCB contaminated rice oil in Japan in the late 1960s developed a condition termed "Yusho". A similar case of PCB poisoning occurred in Taiwan in 1979 which was termed "Yu-Cheng", resulting in the same symptoms seen in Japan. Yusho and Yu-Cheng patients had levels of PCBs reported in the 50 ng/ml range as well as polychlorinated dibenzofurans (PCDFs) at levels around 0.1 ng/ml in their blood (*50*). Children of exposed Yusho and Yu-Cheng mothers developed distinct clinical manifestations including dark brown skin and mucous membrane pigmentation, edematous eye, dentition at birth, abnormal skull calcification, and low birth weight (*51*). The effects seen in these children demonstrates the teratogenicity of organochlorines such as PCBs and PCDFs.

Finally, the Inuit population is exposed to large amounts of PCBs through a diet rich in seals and other marine mammals which are near the top of the Arctic aquatic food chain (*52*). These animals themselves have bioaccumulated levels of PCBs as high as 8μg/g of lipid, which are stored at highest levels in the blubber at 1.9 μg/g (wet weight) and are also detected in other tissues consumed as meat at 0.8 μg/g (*53*). Inuit women in northern Québec have approximately 7 μg/g of lipid of PCBs in their breast milk, which is 7 fold higher than that found in Caucasian women in southern Québec (*52*).

1.2.3 Health Effects of PCBs

PCBs have a wide range of health effects on different systems depending on the type of PCB, the amount of PCB in the exposure, the length of exposure, and the organism exposed (*37*). The more highly chlorinated PCBs tend to bioaccumulate more and have a lower rate of metabolism compared to lower chlorinated PCBs. This is well demonstrated in a study of the tissues of rats dosed with Aroclor 1254. Relative to Aroclor 1254, the brain, liver, blood, and adipose tissue contained a lower concentration of tetra- and penta- congeners, and a greater concentration of heavily chlorinated congeners in the hexa- to nona- range (*54*). The more PCBs that enter the body, the more harmful effects that may be seen. Although these compounds have high LD_{50}s of approximately 1300 mg/kg (depending on the components of the PCB mixture), some effects due to acute exposure are more severe than those due to chronic exposure. The bioaccumulative nature of PCBs is also a factor regarding the length of exposure. An individual will accumulate PCBs in their tissues during the time they are exposed and some of these PCBs will remain for months or years without being metabolized. PCB toxicity studies have been performed in various organisms including mice, rats, mink,

guinea pigs, and monkeys. In some studies mink and monkeys exhibit a higher degree of sensitivity to PCBs than other species such as mice, rats, and rabbits, particularly regarding the influence of PCBs on body weight (*55, 56*). Variation of PCB effects in different species may be modulated by species-specific discrepancies in lipid metabolism, enzyme induction as well as quantitative differences in PCBs binding to receptors in target organs (*57*). The toxicity of PCBs in humans has only been measured in individuals exposed through occupational or accidental exposure. Therefore, we must also rely on evidence of these effects in animal studies to suggest how human systems may be affected.

PCBs have caused renal damage in animal studies. Rats treated with 100 mg/kg/day of PCBs for 3 days per week for 3 weeks developed renal tubular damage, including vacuolated tubular epithelial cells with fatty deposits, and epithelial cells and proteinaceous casts in the tubular lumens and urine (*58*). No significant effects on kidney weight has been reported (*59*).

Dermal effects due to PCB exposure has been reported in humans as well as laboratory animals. The levels of PCBs in these exposures range from less than 0.1 mg/m^3 to 11 mg/m^3 over at least 4 months in the humans and as low as 0.1 mg/kg/day in monkeys fed PCBs for 2 months (*59*). The symptoms include facial edema, chloracne, alopecia, thickening and pigmentation disturbances of the skin and nails, and fingernail loss (*60-64*).

The immune system has also been shown to be susceptible to the effects of PCBs. Intermediate duration exposure studies have induced a decrease in serum levels of immunoglobulins and an increase in susceptibility to bacterial infections in mice treated

with 22mg/kg/day of PCBs for 3 to 6 weeks (*65-67*). PCBs have also been shown to cause similar effects in monkeys (*68, 69*).

Neurological effects of PCBs have been reported in humans and animals. Individuals such as the Inuit with a diet high in fish from PCB contaminated waters and those consuming fish from the Great Lakes are susceptible to these effects. PCB exposure has been correlated to changes in neuropsychological functioning in groups of people with a diet rich in fish (*70*). Studies of PCB effects at different stages of development in humans and rodents show alterations in dopamine levels (*59*). PCBs can also affect memory, attention, and learning in humans and monkeys exposed *in utero* and through lactation (*71, 72*).

Although PCBs can effect all systems in the body, one of the most notable and consistent effects is on the liver. PCB doses above 0.3 mg/kg/day for 3 to 15 weeks have been shown to cause increased liver weights in rats (*44, 73-78*), although biochemical alterations were not observed in all of these studies. The lowest observed adverse effect level (LOAEL) for PCB induced hepatic effects in mice is 200 mg/kg, and the no observed adverse effect level (NOAEL) is a single dose of 50 mg/kg. Histological effects of PCBs in rats are seen at doses of 50 mg/kg/day for 30 days and 11 months and of 5 mg/kg/day for 6 months. The effects from these studies include hepatocyte hypertrophy, fat deposition, fibrosis, necrosis, and changes in serum levels of liver associated enzymes indicative of possible hepatocellular damage (*59*).

PCBs are known to have effects on induction of enzymes in the cytochrome P450 (CYP) system. CYP and MFOs involved in PCB metabolism are primarly located in the liver (*79*) and are involved in the biosynthesis and catabolism of steroid hormones as well

as the detoxification of foreign compounds that enter the body. The presence of chlorine substitutions on the carbon atoms in a PCB molecule blocks metabolism at the position of substitution. MFOs in the liver metabolize PCBs to arene oxide intermediates, which undergo oxidationto polychlorinated hydroxybiphenyls, followed by spontaneous or epoxide hydrolase mediated reduction (*80*). PCBs that have been hydroxylated or sulfonated by phase II enzymes are known to bind to steroid hormone receptors (*81-85*) and to be estrogenic (*85, 86*). CYP 1A and 2B enzymes are both thought to be involved in PCB metabolism (*87*). One of the intriguing aspects of PCB action on metabolism is that they induce the production of the enzymes that metabolize them. Aroclor 1254 is known to stimulate the expression of CYP enzymes including some of the CYP 1A, 2A, 2B, 3A enzymes. Epoxide hydrolase, glucoronosyl transferase, glutathione S transferase, and some reductases are also induced by Aroclor 1254 (*88*).

In addition to effects on specific organ systems in the body, PCBs can affect body weight. In animal studies, weight loss after a single high dose was attributed to dehydration (*58*). Intermediate and chronic duration dietary administration of PCBs often causes a decrease in body weight or body weight gain, which is considered to constitute a wasting syndrome (*59*).

1.3 Endocrine Disrupting Characteristics of PCBs

1.3.1 *In vitro* Evidence

A common method used to assess the estrogenic effects of a compound is the E-SCREEN assay. This method tests the estrogenicity of environmental chemicals by measuring the proliferative effect on MCF-7 cells (*86*). This study found five PCB congeners as well as some of the hydroxylated metabolites of some congeners to be estrogenic. Other studies examined the binding affinity of hydroxylated PCB congeners

and found the most potent congener was 2,4,6,2',6'-pentachloro-4-biphenylol, which was approximately 5 times less potent than 17ß-estradiol (*89*). The congeners found to be estrogenic were not coplanar, ie. they did not have the two benzene rings in the same plane. The degree of planarity is determined by the presence of ortho-substituted chlorines, which cause steric hindrance to rotation. Coplanar PCBs have one or less ortho-substituted chlorine atoms (*90*). Coplanar PCBs are thought to act through the aryl hydrocarbon receptor (AhR) and exhibit dioxin-like characteristics, and are the most toxic PCBs when assessed for their TCDD equivalency factor (TEF) (*57*). Transcriptional assays have found some hydroxylated PCB congeners bound to the ER and altered gene expression mainly in an antiestrogenic manner and decreased reporter gene expression (*82, 91*). There have been no previous reports demonstrating the ability of PCBs to bind to the thyroid hormone receptor (TR) or AR *in vitro*.

1.3.2 *In vivo* Evidence

Studies of endocrine disruption by PCBs have examined a number of hormonal systems and have been conducted in animals and in humans through epidemiological analysis. A study was conducted on the effects of PCBs on turtles that utilize temperature-dependent sex determination (common in many egg-laying reptiles). The investigators found that in turtle eggs incubated at male-producing temperatures, the estrogenic effects of PCBs reversed the gonadal sex to female (*92*).

Many epidemiological studies on breast cancer and environmental exposure to PCBs have been conducted. The previously mentioned *in vitro* studies on the estrogenicity of PCBs suggest that these compounds are contributing factors to high rates of breast cancer in industrialized countries. However, the relationship between PCBs and

breast cancer remains unclear. Some studies show a significant relationship involving a correlation between breast adipose tissue levels of PCB congeners and breast cancer risk (*93*), and the ability of PCBs to produce free radical-mediated oxidative DNA damage during oxidation of lower chlorinated biphenyls (*94*). The detection of increased oxidative damage of DNA in human breast tumour tissue suggests that this area warrants further investigation. Another study found that PCBs are present in breast cyst fluids and therefore in contact with the breast ductal epithelium (*95*). Despite this evidence, other epidemiological studies have found no correlation between serum PCBs and breast cancer risk (*96-98*).

Another component of the endocrine system that is markedly affected by PCBs *in vivo* is the thyroid hormone system. Studies of rats treated with 0.2 to 1.8 mg/kg (*99*) and 5 to 50 mg/kg/day for 5 to 7 months (*100*) found that serum and plasma triiodothyronine (T3) and thyroxine (T4) levels were suppressed in a dose-related manner in neonatal, pregnant, and adult rats. This reduction results from direct thyroid damage, rather than an increase in hepatic catabolism (*100*). As thyroid hormones are involved in fetal brain development, these observations hold important relevance regarding the role of thyroid hormones in brain maturation and the developmental neurotoxicity induced by PCBs (*99*). Furthermore, PCBs have been shown to reversibly increase Sertoli cell proliferation and testis weight through suppression of T4 in rats treated with 1.6 and 3.2 mg/day during gestation (*101*). In addition to inducing hypothyroidism, 50 and 500 mg/kg of PCBs administered to rats translactationally as well as directly via the oral route produced ultrastructural lesions such as an increased development of rough endoplasmic reticulum and mitochondrial vacuolization in thyroid follicular cells. This contributed to

decreases in serum thyroid hormone levels (*102*). The decrease in serum thyroxine levels in this case is attributed to an interference with hormone secretion and an enhanced peripheral metabolism of thyroxine (*103*).

Evidence of decreased serum levels of adrenal cortex hormones has also been reported in animal studies. Alterations in adrenal function including decreased serum corticosterone levels in rats treated orally with 1 to 50 mg/kg/day PCBs for at least 5 months has been reported (*104*). By contrast, an increase in serum corticosterone levels after treatment with at least 8.1 mg/kg/day of PCBs for 2 weeks has been observed in mice (*105*). However, a study of monkeys exposed to 0.08 mg/kg/day of PCBs for 22 months showed no change in serum hydrocortisone (*106*). These discrepancies may be due to species-specific differences in principal glucocorticoids and the effects of PCBs on the metabolism of glucocorticoids in each species (*59*).

1.3.1.1 Female Reproductive Effects

PCBs were uterotropic when administered to immature female rats in doses of 30 and 120 mg/kg (*107*). PCBs at 0.1 to 10 µg/ml also decreased the *in vitro* fertilizing ability of exposed mouse oocytes (*108*). Monkeys treated with 20 and 80 µg/kg/day PCBs for about 2 years experienced changes in luteal phase progesterone levels and a marginally longer duration of menses (*109*). Neonatal female rats exposed to 110 µmol/kg PCBs exhibited increased hepatic basal testosterone hydroxylase activity, androstenedione formation, and testosterone metabolism (*110*). Female offspring of rats exposed to PCBs as low as 8 µg/kg during lactation underwent a delay in puberty. When treated with doses between 32 and 64 µg/kg these rats had decreased uterine wet weight, and offspring in the 64 µg/kg group exhibited impaired fertility and irregular estrus cycle patterns (*111*).

Endocrine modulating effects related to estrogen have also been observed in humans. Women who consumed PCB contaminated fish had a significant reduction in menstrual cycle length (*112*). Another study found high levels around 790 ng/ml of some PCB congeners in the blood of women with repeated miscarriages which correlated with immunological and hormonal changes, such as a decrease in testosterone levels (r_s=-0.23126) (*113*).

1.3.1.2 Male Reproductive Effects

Exposure of sperm to a capacitation medium containing PCBs did not effect *in vitro* fertilizability or sperm motility in mice (*108*). However, male rats treated with 100 μmol/kg PCBs neonatally exhibited decreased hepatic basal testosterone hydroxylase activity and androstenedione formation (*110*). The male offspring of rats dosed with 8, 32, and 64 mg/kg/day PCBs for 6 days during lactation developed significantly smaller prostates with fewer acini and altered morphology of the epithelial cells compared to controls in adulthood (*114*). Studies have also shown rats treated during lactation and/or *in utero* developed larger testes (*101, 114*). A reduction in seminal vesicle and epididymal weights, and caudal epididymal sperm counts has been reported (*44*) in rats treated during development. These studies indicate some of the potential reproductive target sites of PCBs in males; however, these findings are not consistent with other reports (*44, 115*). The effects of PCBs on male reproductive organs such as the prostate, epididymis, and testis are examined in this study.

1.4 The Prostate

1.4.1 Structure and Function

The prostate is an exocrine gland that surrounds the urethra immediately below the bladder. This organ functions to produce secretions containing proteins and proteases

from the ducts in the prostate. Aliphatic polyamines such as spermidine and spermine are components of the secretions which are discharged into the urethra and may play a role in reducing the rate of seminal clot formation during ejaculation (*116*). In humans, the prostatic ducts originate from the urethra and radiate peripherally to completely surround the urethra. In rats and mice, the ducts are organized and encapsulated into individual lobes around the urethra (*117*).

1.4.2 The Androgen Receptor and Hormonal Regulation

The androgen receptor (AR) is a member of the superfamily of nuclear receptors that act as transcription factors. Once the receptor binds to the ligand, this complex binds to DNA regulatory elements and activates gene transcription for specific genes. The receptors in this superfamily have structural and functional characteristics in common. The AR belongs to a subgroup of steroid hormone receptors that also includes the ER, GR, progesterone receptor (PR), and mineralocorticoid receptor (MR) (*118*). In the steroid receptor family, there are several conserved functional domains, such as a transactivating N-terminal domain (TAF_1), a DNA-binding domain (DBD), a hinge region involved in nuclear translocation and dimerization, and a C terminal ligand binding domain (LBD) which also has a transactivating function (TAF_2) (*119*) (Figure 4).

The prostate is an androgen target organ for its growth, development, and differentiation, and is responsive to androgens during fetal, pubertal, and adult stages (*117*). The secretory epithelial cells in the prostate require androgens, which are produced in the testis to maintain normal structure and function. The removal of androgens causes an inhibition of cellular proliferation and an induction of cell death mechanisms, which results in involution of the prostate (*120*). The development of

carcinoma of the prostate is an androgen dependent process (*121*). Prostate cancer is the most commonly diagnosed lethal cancer in Canadian men and has become the second greatest cause of cancer deaths in Canadian men (*122*).

1.4.3 Xenobiotic Effects on the Prostate

There are a variety of components which contribute to the patterns of prostate cancer occurrence observed in epidemiological studies. These include age, ethnic background, country of residence, and diet (*123*). Genetic factors also contribute to the development of this disease. An individual in North America with a first-degree relative who is affected has a 2 fold increased risk of developing prostate cancer (*124*). Genes linked to prostate cancer have also been found, including HPC1 on 1q (*125*), and some genes on the X chromosome (*126, 127*). It has been proposed that high incidence of this disease in industrialized countries may be influenced by environmental factors (*128*). As these environmental contaminants can interfere with normal androgen function, the changes that could occur in all stages of life may aid in the development and progression of prostate cancer. A statistically significant association was found between prostate cancer deaths and the number of acres that Canadian farmers sprayed with herbicides (*129*). This suggests a possible role of chemical exposure, perhaps through endocrine disrupting mechanisms in the etiology of adenocarcinoma of the prostate (*130*).

The hormonal sensitivity of the prostate makes it vulnerable to endocrine disrupting effects, and the prostate is considered one of the most effected sites in the male reproductive endocrine system (*130*). This sensitivity is demonstrated in part by the influence of estradiol on prostate development *in utero*. Neonatal exposure to estradiol caused a decrease in AR expression in prostatic cells and significantly retarded growth

and epithelial cytodifferentiation, which demonstrates that basal epithelial cells are a possible target of androgen action during prostate morphogenesis (*131*). In addition, a low concentration of free serum estradiol during murine fetal life causes an increase in the number of developing prostatic glands during fetal life. In adulthood these mice had an increase in the number of prostatic ARs per cell and the prostate was enlarged due to hyperplasia. Mice fetuses exposed to high concentrations of estradiol experienced a decrease in prostate weight in adulthood (*25*). Interestingly, murine fetal exposure to low doses of the xenoestrogen DES caused an increase in prostate weight in adulthood (*132*). In addition, male rats exposed postnatally to PCBs developed histological alterations in the ventral prostate (*133*). These studies demonstrate the sensitivity of the prostate gland to hormones and xenobiotics during development and the possibility of long term effects such exposures can produce.

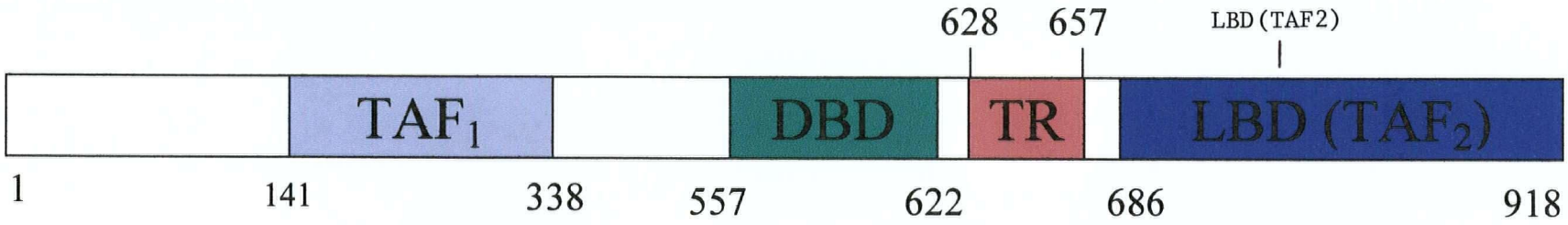


Figure 4. Functional domains of the androgen receptor. These domains include the transcriptional activation domain (TAF_1), DNA binding domain (DBD), hinge region/nuclear translocation signal (TR), and the ligand binding domain (LBD (TAF_2)). Diagram adapted from Keller, E. *et al.*, 1996.

1.5 Rationale and Objectives of this Study

The influence of organochlorine environmental contaminants such as PCBs on biological systems can be severe and deleterious to all organisms in the ecosystem. Although PCBs are no longer manufactured and large releases of PCBs are prohibited in industrialized countries, due to their chemical properties and bioaccumulative nature, PCBs are still found in organisms at all trophic levels. These compounds are known to have endocrine disrupting capabilities. Most studies of the effects of PCBs on hormonal systems have focused on estrogen and thyroid hormone as targets. Indeed, endocrine disruption as a field of research has had great emphasis historically on estrogen mimics and environmental estrogens. Only a few studies have shown that environmental contaminants can block or increase the activity of androgens at the molecular level and affect reproductive development. The role of PCBs as androgenic endocrine disruptors has not been extensively studied. In order to further assess the impact of PCBs on the androgen axis, analysis of AR interactions and alterations in prostate growth and development are warranted.

Hypothesis: The hypothesis to be tested is that PCBs have the ability to alter androgen regulated processes *in vitro* and *in vivo*.

Objective 1. To test the influence of four different PCB mixtures, Aroclors 1242, 1248, 1254, and 1260 on androgen-regulated reporter gene expression in the LNCaP prostate cell line. Also to examine the influence of these chemicals on glucocorticoid-regulated reporter gene expression in order to demonstrate the presence of any androgen-specific effects.

Objective 2. To determine the effects of two congeners, 2,3,4, trichlorobiphenyl (PCB 31) and 2,2',3,4' tetrachlorobiphenyl (PCB 42) in the same system

described above in order to gain insight into the action of individual congeners that are present at different concentrations in each mixture.

Objective 3. To test the ability of these mixtures and congeners to interfere with binding of DHT (a natural AR ligand) in a cell line stably transfected with a FLAG-tagged AR.

Objective 4. To evaluate the effects of PCBs on the prostate in the mouse model transgenic for a prostate-specific, androgen sensitive reporter gene. This animal model was used to assess the ability of the PCB mixture, Aroclor 1254, to interfere with specifically androgen regulated gene expression *in vivo* and the alterations in prostate size and development that occur as a result of PCB exposure.

CHAPTER 2.

EXPERIMENTAL PROCEDURES

2.1 *In vitro* Studies

2.1.1 Chemicals

PCBs were produced for industrial purposes and sold commercially as mixtures under a number of names, including Aroclor. These mixtures are composed of up to 209 congeners, although some congeners are only detectable at trace levels in some Aroclor mixtures. Four Aroclors, 1242, 1248, 1254, and 1260 were tested in this study. These four digit identifiers shows the parent molecule is a 12 carbon backbone by the first two digits in the identifier (12) and the last two digits indicate the overall chlorine content by weight percent of the entire mixture (*90*). The individual PCB congeners tested in this study were 2,3,4, trichlorobiphenyl (IUPAC PCB 31) and 2,2',3,4' tetrachlorobiphenyl (IUPAC PCB 42). These congeners are present in the Aroclor mixtures at different levels (Table 2.1). PCB 31 is 4.5% of Aroclor 1242, 9.3% of the content of Aroclor 1248, 0.7% of Aroclor 1254, and is not detectable in Aroclor 1260. PCB 42 is not detectable in Aroclor 1242, comprises 7% of Aroclor 1248, 2.2% of Aroclor 1254, and 0.7% of Aroclor 1260. Thus, these congeners represent a distinct component of each mixture, but exert their influence on Aroclor mixture action at different levels.

Aroclor 1242 (mol wt.=266.5), Aroclor 1248 (mol wt.=299.5), Aroclor 1254 (mol wt.=328), Aroclor 1260 (mol wt.=375.7), PCB 31, and PCB 42 were purchased from Ultra Scientific (North Kingstown, RI). Compounds were dissolved in absolute ethanol (Aroclors) or hexane (PCB 31 and 42) for a 0.1 M stock.

Table 1. Percent content by weight of some individual congeners in four Aroclor mixtures. * indicates congeners tested in this study.

	Aroclor			
Congener IUPAC #	1242	1248	1254	1260
31*	4.53	9.31	0.72	trace
42*	trace	7.05	2.18	0.66
45	0.9	5.73	0.15	trace
66	0.81	4.95	2.24	0.22
99	0.55	2.52	6.1	0.82
101	0.27	1.5	6.98	5.04
118	trace	trace	8.09	2
168	trace	0.56	4.23	0.59
193	trace	trace	2.3	trace
198	trace	trace	1	0.15

For cell culture studies serial dilutions were prepared in absolute ethanol with a final concentration of ethanol of 0.1% in culture. The final concentration of ethanol in the DHT and dexamethasone (DEX) (Steraloids Inc., Pawlings, NY) were dissolved in absolute ethanol for 1 mg/ml stocks. Serial dilutions of DHT and DEX were prepared fresh for each experiment. These dilutions were in 20% ethanol, for a final ethanol concentration of approximately 0.2% in culture.

2.1.2 Plasmid Constructs

The cell culture transfection assays utilized four different plasmids. The first plasmid contained the full length cDNA of the rat AR driven by the CMV promoter; the plasmid has been named pAR6CMV (*134*).

The rat GR plasmid was obtained from Dr. R.J. Matusik. This construct consists of a 2.8 kb insert containing 24 nucleotides upstream of the rat GR primary translation initiation site to 360 nucleotides downstream of the termination codon and is driven by the double RSV-SV40 promoter; this plasmid is named prGR (*135*).

The reporter used was the ARR_3tk-luc which contains three tandem repeats of -244 to -96 of the promoter region of the rat probasin gene (*136*). This promoter region contains androgen response elements to which both AR and GR bind to and transactivate from *in vitro* (*in vivo*, the probasin promoter is regulated by the AR endogenous gene only). The promoter is ligated in the pT81 vector (American Type Culture Collection, Rockville, MD), which contains a minimal thymidine kinase promoter, and the firefly luciferase gene, as described previously (*134*). The firefly luciferase gene is translated to a 61 kDa monomeric protein that does not undergo post-translational modification for

enzyme activity and is a genetic reporter that functions immediately upon translation (*137, 138*).

An additional plasmid was used as a transfection efficiency control. The pRL-TK transfection control vector contains a thymidine kinase promoter upstream of Rluc. Rluc is the slightly modified cDNA encoding Renilla luciferase from the sea pansy *Renilla reniformis* (Promega, Madison, Wisconsin).

All plasmid DNA was propagated in JM109 *E. coli* and was prepared using QIAGEN Maxiprep Kit (QIAGEN, Mississauga, ON, Canada).

2.1.3 Cell Culture Transfections

LNCaP cells originated as a lymph node metastasis from the prostate in a 50 year old Caucasian male. These cells are well differentiated androgen-sensitive prostate cells which express an endogenous mutated yet functional AR with an alanine to threonine mutation at position 868 (*139*). LNCaP cells were maintained in RPMI 1640 defined medium (GibcoBRL, Burlington, ON, Canada) supplemented with 5% fetal bovine serum under standard conditions (37°C, 5% CO_2). For transfections, cells were cultured in RPMI 1640 defined medium supplemented with 5% hormone stripped (with dextran coated charcoal) fetal bovine serum (S-FBS) at a density of 3x 10^5 cells/well in 6 well cell culture plates (Costar, Corning NY) and incubated overnight under standard conditions. Plasmids were cotransfected into the cells using LIPOFECTIN Reagent (GibcoBRL, Burlington, ON, Canada). Each plate received 1.5 μg of the AR or GR plasmid, 0.01 μg of $pARR_3tk$-luc, and 0.005 μg of pRL-TK. The cells were incubated overnight and hormone and PCB were added simultaneously. Cells were harvested 48 h later in PBS with 1mM EDTA and microcentrifuged at 3000 rpm at 4°C for 4 min.

Supernatant was removed and 0.1 ml Passive Lysis Buffer (Promega, Madison, WI) was added. Cells remained on ice for 15 min and were then gently vortexed and frozen at –80°C until analysis. Luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI) and the EG&G Berthold Microplate Luminometer LB 96V.

An average of at least two experimental determinations with three replicates each were done for each PCB and hormone combination to give at least six samples per group. Firefly luciferase values were normalized for transfection efficiency using the activity of Renilla Luciferase as baseline levels. Values were presented as mean of total replicates (minimum of 6) corrected for background and control value ± standard error of the mean (SEM).

2.1.4 Cell Culture Ligand Displacement Assay

HeLa cells expressing a stably transfected FLAG-tagged AR were obtained from Dr. M. Carey (*140*). The FLAG-AR fusion protein is an aspartate rich octamer at the N terminus of the AR. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco BRL, Burlington, ON, Canada) supplemented with 10% S-FBS at 30 x 10^5 cells/plate in 10 cm^2 plates (Costar, Corning, NY). Cells were incubated for 5 h in standard conditions. Media was changed to DMEM supplemented with 10% S-FBS, 0.3 nM [1,2,4,5,6,7-3H(N)]-DHT (NEN Life Science Products, Inc., Boston, MA, specific activity=123 Ci/mol, initial concentration=1mCi/ml) in 20% ethanol and PCB in 100% ethanol. Cells were incubated 20 to 24 h and washed with PBS and harvested by scraping. Nuclear extracts (*141*) were prepared and frozen at -80°C prior to use. Briefly, harvested cells were centrifuged for 4 min at 4000 rpm. PBS was removed, and add 4 ml

cold buffer A (10 mM HEPES pH 7.9, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA, fresh 1mM dithiothreitol, fresh 0.5mM phenylmethylsulfonyl fluoride) vortexed carefully and set on ice 15 min. 250 µl of 10% IGEPAL was added, vortexed vigorously for 10 seconds and centrifuged immediately for 1 min at 4000 rpm. Supernatant was removed, and 500µl cold buffer C (20mM HEPES pH 7.9, 0.4M NaCl, 1mM EDTA, 1mM EGTA, fresh 1mM dithiothreitol, fresh 1mM phenylmethylsulfonyl fluoride) was added. Nuclear pellets were resuspended, vortexed vigorously for 15 min at 4°C, centrifuged for 5 min at 4000 rpm at 4°C and frozen. Extracts were thawed and half of the extract was incubated for 6 h with 10 µl of agarose beads conjugated with anti-FLAG monoclonal antibodies (Sigma Chemical Co., St-Louis, MO) at 4°C for 6 h. Beads were washed 2 times with Buffer D (*140*) containing 20mM HEPES pH 7.9, 20% glycerol, 0.3M KCl, 0.2mM EDTA, 0.05% IGEPAL, 0.5mM dithiothreitol, 0.5mM phenylmethylsulfonyl fluoride, and then resuspended in buffer D. Resuspensions were measured for levels of tritium by scintillation counting in a Beckman LS 6500 scintillation counter.

Western Blotting was performed according to the method of Harlow and Lane, (*142*) using the remaining portion of the extracts. Thawed nuclear extracts were heated at 100°C for 5 minutes in 2X sample buffer (0.125M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 5% mercaptoethanol), and put on ice and then loaded into gels. Samples were electrophoresed on 10% SDS-PAGE minigels and transferred to PVDF membranes overnight. After transfer, membranes were washed 2X for 5 minutes in TBST (100mM Tris-HCl pH 7.5, 0.9% NaCl, 0.1% Tween-20) and blocked with 10% dried skim milk in TBST for 1 hour at room temperature. The primary antibody, an anti-mouse monoclonal antibody to human AR DBD (Pharmingen, Mississauga, ON, Canada) was added to the

blots in TBST (1:500) for 2 hours. Membranes were washed 3 times for 10 minutes with TBST. Following the washes, membranes were incubated with anit-mouse-HRP (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:5000. Membrane bound antibodies were detected using chemiluminescence with ECL™ Western blotting detection agents (Amersham Pharmacia Biotech Inc., Baie d'Urfé, Québec) for an incubation period of 30 to 60 s, and exposed immediately to scientific imaging film (Kodak Eastman Co., Rochester, NY). Films from Western blots were analyzed using BioRad Quantity One Quantitation Software (BioRad, Hercules, CA) to determine and compare band densities for nuclear extracts from each ligand displacement assay.

Values in this assay are presented as the amount of ^{3}H-DHT (DPM) bound to the AR and corrected for the amount of AR present in the sample (dividing by the value calculated as the density of the band after Western blotting).

2.2 *In vivo* Studies

2.2.1 Animals

This study used CD1 transgenic mice possessing the androgen sensitive LBP-CAT reporter gene (*143*). This transgene is comprised of a large fragment of the rat probasin promoter (-11500 to +28 bp) (LPB) linked to a chloramphenicol acetyl transferase (CAT) gene to achieve highly prostate-specific expression of CAT in the prostatic secretory epithelial cells. Expression is highest in the ventral prostate, and therefore, this lobe of the prostate was used for biochemical assays. Sexually mature LPB-CAT mice were combined in mating pairs and females were treated daily by gavage. Four to five pairs were used for each treatment group to ensure a minimum of five male pups per group. Females were checked daily for copulatory plugs. Males were removed on approximately day 18 of gestation to prevent re-impregnation after birth of

the litter. Females, litters, and male weanlings were weighed daily. All mice were fed Lab Diet 5015 (PMI Foods, Richmond, IN) and water *ad libitum* and were kept on a cycle of 12 hours light followed by 12 hours darkness at 20-25°C. These animal studies were conducted according to the Canadian Council of Animal Care Guidelines and were reviewed by the University of British Columbia Animal Care Committee.

2.2.2 Treatment and Groupings

Treatments used were 10 mg/kg/day Aroclor 1254 (Ultra Scientific, North Kingstown, RI) in 0.1 ml canola oil, and 0.1 ml canola oil (Safeway Brand, Safeway Canada) for the control in the single dose level group. The dose response group received 10, 20, or 40 mg/kg/day Aroclor 1254 in 0.2 ml canola oil and 0.2 ml canola oil (Ultra Scientific) for the control group. Females were dosed until weaning when the pups were 21 days old. Weaned male pups from the litter were treated via gavage daily until the ages of 28 days old (single dose level group only) and 56 days old. At the end of the treatment the animals were sacrificed by cardiac exsanguination under Metofane anesthesia (Janssen, Toronto, ON, Canada). The left epididymis, left testis, and left kidney were removed, weighed and frozen. Organ weights were calculated as organ weight/body weight in order to correct for mouse to mouse variation in body weight. This data is presented as group mean ± SEM.

The right epididymis and right testis were removed and fixed in 10% neutral buffered formalin (NBF) for histology. The liver, heart, and ventral prostate were removed, weighed, subdivided and half frozen. The remaining half was fixed in 10% NBF.

The doses were selected based on the solubility limits of Aroclor 1254 in canola oil of 1 mg/ml, and on the basis of toxic levels reported for the liver, as PCBs are known

to cause severe hepatic effects. A dose of 50 mg/kg/day of PCBs administered to rats for 30 days resulted in degenerative histological liver changes (*144*). A dose of 2.5 mg/kg/day of PCBs administered to rats for 5 months did not cause any significant changes in liver weights or histology (*104*). Therefore, doses used in our study were within this range of level and duration of treatment.

2.2.3 CAT Assay

Frozen tissues were thawed on ice and homogenized in 300 µl lysis buffer (0.1M Tris, pH 7.8, 0.1% Triton X-200) with 10 strokes of a Pyrex Tenbroeck Glass homogenizer. Extracts were removed from homogenizer and centrifuged at 8000 rpm for 3 minutes at 4°C. Supernatant was assayed for protein concentration using the Pierce BCA protein assay kit (Rockford, Illinois) and results read at 560 nm in a Titertek Multiskan platereader (Flow Laboratories, Mississauga, ON, Canada). Aliquots containing 0.1 to 1 µg of protein from each sample in duplicates in 200 µl lysis buffer were heated at 65°C for 10 minutes and cooled at room temperature for 10 minutes. A reaction mixture combining ^{3}H Acetyl-Co A, 5mM Chloramphenicol, and 1M Tris pH 7.8 was added to the sample. 3 mL of ScintiLene (Fisher Scientific, Whitby, ON, Canada) was added and incubated at room temperature for 30 minutes. Samples were read in a scintillation counter for 1 minute per sample for 5 cycles. Slope of the line from the five cycles was calculated and converted to dpm/min/mg protein for the CAT activity. CAT activity was presented as mean ± SEM.

2.2.4 Histology

Fixed tissues were paraffin embedded and slides prepared at VGH Pathology Laboratories. All slides were H and E stained. Sections were then assessed for

histological anomalies by a veterinary pathologist (Stéphane Lair). A photonic microscope was used. The parameters examined include evaluation of different histological structures and grading of the distribution of such structures in each section. Other histological findings such as anomalies in the tissues were also reported and graded.

2.2.4.1 Measurement of Percent Area of Stroma and Ducts in Prostate Tissue Sections

Three photographs of random areas of stained slides of prostate tissue from each experimental animal were captured using CoolSNAP software connected to an inverted microscope (Leica, Wetzlar, Germany). Each captured image was exported to Adobe Photoshop 5.5. Drawing tools in this program were used to cover all the ductal structure in the image with the colour red. The remaining area in the image was the stroma. This area was coloured white. The number of red and white pixels relative to the total number of pixels was calculated in Photoshop to give a representative value of the percent area for stroma and ducts. A ratio of stromal to ductal area was also calculated based on the number of pixels assigned to each type of area. The percent area for each image was averaged with others for the same treatment group for comparison. This calculation was used to determine if the treatment related increase in dilated acini was due to a change in the stromal or ductal area, which may account for the decrease in prostate weight with increasing treatment dose.

2.2.5 Measurement of Serum Testosterone Levels

Serum collected by cardiac exsanguination was frozen at –20°C. Samples from the single dose level group that were in excess of 200 µl were sent to the Tumour Marker

Laboratory at the British Columbia Cancer Agency for total testosterone radioimmunoassay (DPC, Los Angeles, CA). The sensitivity of this assay is at 0.14 nmol/l, and the upper limit of the range of detection is 56 nmol/l. Crossreactivity with DHT is less than 5% and the antiserum is considered highly specific for T. Results were provided in duplicate in nmol/l, and were presented in a scatter plot by treatment group.

2.2.6 Statistics

Statistical analysis for results from the 10 mg/kg/day single dose level group CAT assay and organ weights were analyzed using the Student's *t*-test. The one way analysis of variance (ANOVA) and Dunnett's test for multiple comparisons of means were used for the dose response study and the cell culture ligand displacement assay. $p< 0.05$ was considered significant. The serum testosterone levels data set was analyzed by one way ANOVA as well as by the Kruskal-Wallis test using JMP® (SAS Institute Inc.).

CHAPTER 3.

RESULTS

3.1 Induction of Luciferase Reporter Gene

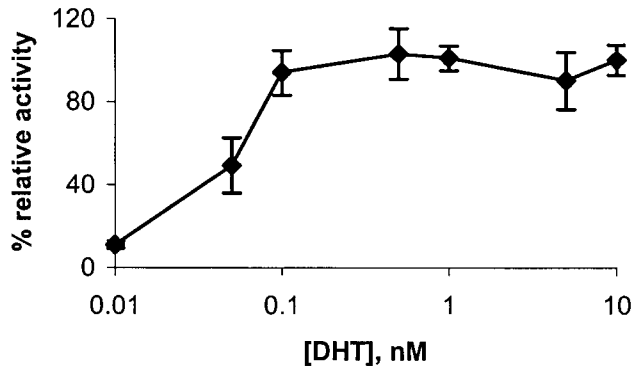
An endocrine disruptor may act as an AR agonist by directly activating androgen receptor function in the absence of hormone, or DHT. In the presence of DHT, there is competition for the receptor ligand binding sites between DHT and the endocrine disruptor, which may result in an antagonistic effect. These same mechanisms for changes in receptor activation may also be seen with the GR and DEX.

This cell culture assay of PCB alteration of steroid hormone action involved transient transfection of LNCaP cells, a well differentiated prostate cancer cell line. Cells were transiently cotransfected with plasmids containing the AR or GR, and the ARR_3tk-luc hormone response elements to which the DNA binding domain of both AR and GR bind. pRL-TK was also cotransfected into the cells. This plasmid is transcribed relative to cell proliferation and was therefore an internal control for this assay. The addition of a ligand such as DHT or DEX induced transcription of the luciferase gene from ARR_3tk-luc. Addition of PCBs can alter such transcription if these compounds have the ability to influence AR or GR activity.

3.1.1 Induction by DHT and DEX

To determine if *in vitro* effects on the luciferase reporter gene assay are specific to a certain steroid hormone receptor, both AR and GR were tested. LNCaP cells transfected with the AR and exposed to a titration of DHT concentrations showed a steep increase in luciferase activity from 0.01 to 0.1 nM followed by a leveling off of activity

a.



b.

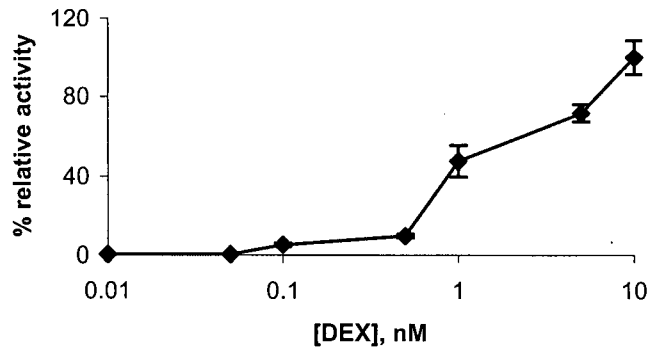


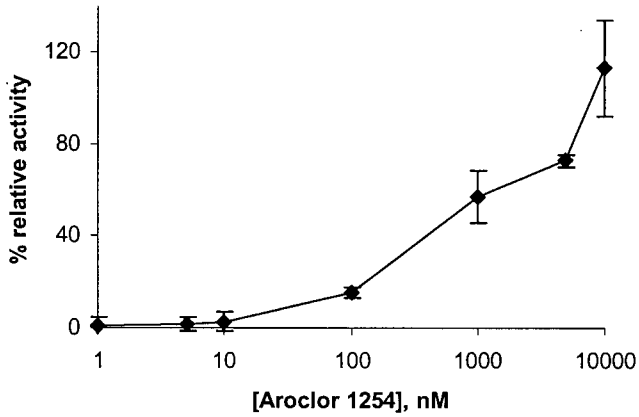
Figure 5. LNCaP cells transfected with a) AR and exposed to DHT titration and b) GR and exposed to DEX titration. Concentration of DHT (a) and DEX (b) in nM is plotted on the x axis, and the percent fold activation with the maximal activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and the maximal activity was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

from 0.1 to 10 nM (Figure 5a). The half-maximal level of peak luciferase activity was at 0.05 nM, and the peak level above 0.1 nM. Therefore, transfected cells were incubated with 0.05 and 1 nM DHT to demonstrate effects in the presence of half maximal and saturating levels of DHT. Cells transfected with the GR and exposed to a titration of DEX concentrations showed low levels of induction from 0.01 to 0.5 nM. Concentrations above 0.5 nM showed a steep increase in DEX induced luciferase activity until 10 nM, although a level of saturation was not reached. The sub-maximal level of luciferase activity from the range tested for DEX was at 1 nM (Figure 5b). Therefore, concentrations of hormone inducing half, sub, and maximal levels were used in combination with Aroclor 1242, Aroclor 1248, Aroclor 1254, Aroclor 1260, PCB 31, and PCB 42 titrations to detect changes in androgen and glucocorticoid driven luciferase activity. In order to measure for AR or GR agonist activity of PCBs, cells transfected with AR and GR were also exposed to titrations of the four Aroclors and two individual congeners in the absence of the known ligands.

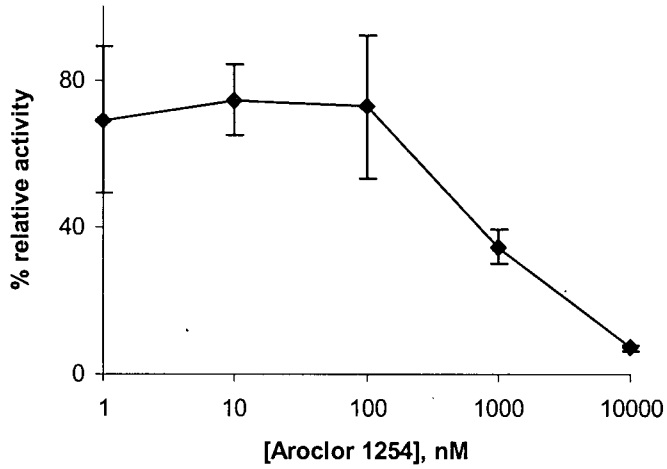
3.1.2 Induction of Luciferase Activity Through AR by Aroclor 1254

In the absence of DHT, Aroclor 1254 (1 to 10 nM) did not induce an increase in luciferase activity. A slight increase was observed at 100 nM, followed by a marked increase in luciferase activity above 100 nM (Figure 6a). In the presence of 0.05 nM DHT there was no change in luciferase activity from 1 to 100 nM, but there was a pronounced antagonistic effect at 1000 and 10000 nM where luciferase activity is reduced by 66% and 93% of the control value, respectively. (Figure 6b). A similar pattern of induction was found in combination with 1nM DHT, where there was a gradual

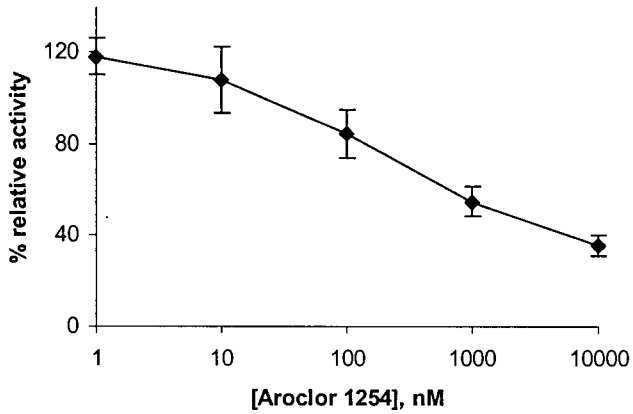
a.


120
80
40
0
% relative activity
1
10
100
1000
10000
[Aroclor 1254], nM

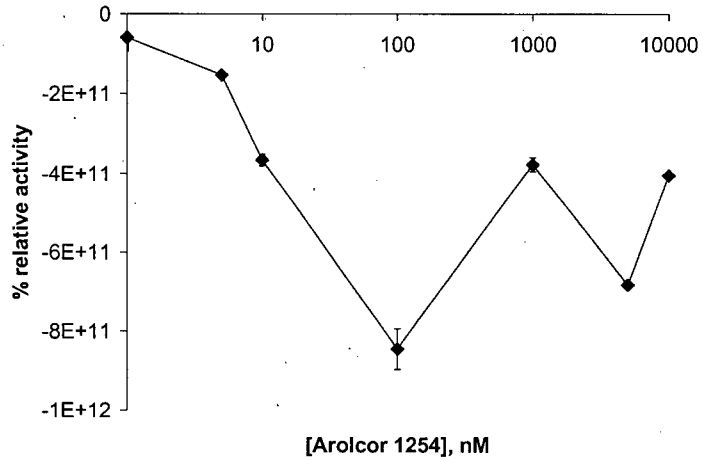
b.


80
40
0
% relative activity
1
10
100
1000
10000
[Aroclor 1254], nM

c.


120
80
40
0
% relative activity
1
10
100
1000
10000
[Aroclor 1254], nM

d.



e.

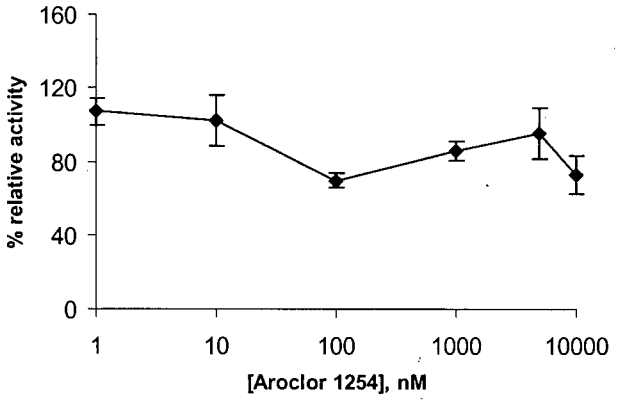


Figure 6. LNCaP cells transfected with AR and exposed to a) Aroclor 1254 titration + no DHT b) Aroclor 1254 titration + 0.05 nM DHT c) Aroclor 1254 titration + 1 nM DHT. Cells transfected with GR and exposed to d) Aroclor 1254 titration + no DEX e) Aroclor 1254 titration + 1nM DEX. Concentration of Aroclor 1254 in nM (b) + 0.05 nM DHT c) + 1 nM DHT e) + 1nM DEX) is plotted on the x axis, and the percent fold activation with maximal activation level set at 100% (a) and the percent fold activation with the control activation level set at 100% (b to e) plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and the maximal activity was set at 100% fold activation for assays without DHT, and control activity (activity before DHT added) was set at 100% fold activation for assays with DHT (not shown on log graph). Data expressed as the mean fold activation ± SEM.

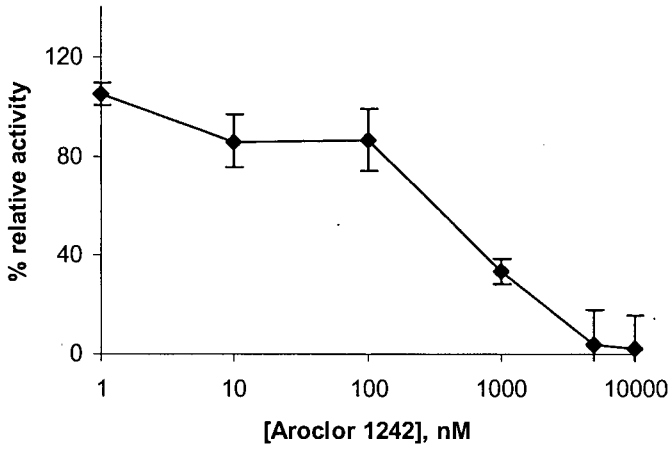
decrease in luciferase activity from 1 to 10000 nM, with a reduction by 64% of control activity (Figure 6c).

These changes suggest that Aroclor 1254 acts as a weak androgen agonist *in vitro*. Aroclor 1254 did not alter luciferase activity either with or without DEX when GR was used to drive the reporter construct. In the absence of DEX, the luciferase induction levels are negative in cells exposed to Aroclor 1254, compared to the levels in the control where no PCB is added (Figure 6d). In the presence of 1 nM DEX, there are no distinct patterns in the trend of the graph (Figure 6e). A 40% drop relative to the control value in luciferase activity is seen at 100 nM, which increases 30% at 5000 nM and drops 30% at 10000 nM. The lack of change in activation of luciferase gene transcription by Aroclor 1254 in cells transfected with GR suggests specificity of Aroclor 1254 for the AR.

3.1.3 Alteration of Luciferase Activity Induction Through AR by Aroclor 1242

Aroclor 1242 did not have a significant effect on luciferase activity with AR in the absence of hormone. In the presence of 0.05 nM DHT there was an antagonistic effect demonstrated by a slight decrease in luciferase activity from 1 to 100 nM, followed by a distinct decrease in luciferase induction above 100 nM with a 98% reduction in luciferase activity at 10000 nM (Figure 7a). There was a similar effect in the presence of 1nM DHT (Figure 7b) where there was no change in luciferase activity from 1 to 100 nM Aroclor 1242, a slight decrease in activity at 1000 nM followed by a steep decrease at 10000 nM by 92%. There was no effect observed with GR in the absence of hormone or in the presence of DEX.

a.



b.

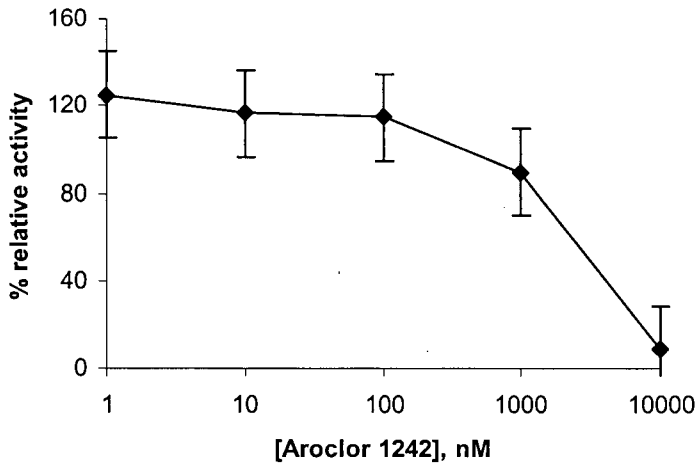
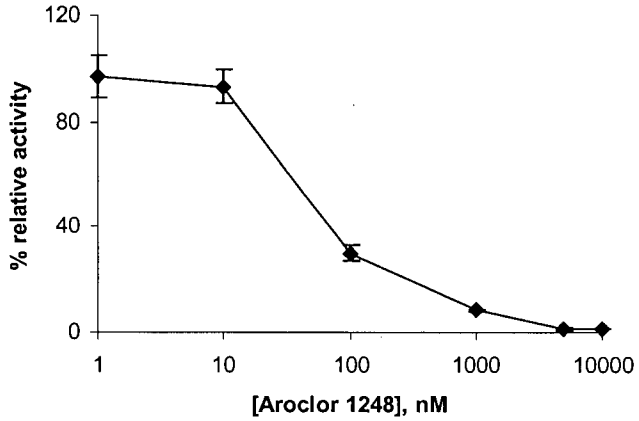


Figure 7. LNCaP cells transfected with AR and exposed to a) Aroclor 1242 + 0.05 nM DHT b) Aroclor 1242 + 1 nM DHT. Concentration of Aroclor 1242 in nM (a)+ 0.05 nM DHT b) + 1nM DHT) is plotted on the x axis, and the percent fold activation with the control activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and control activity (activity before DHT added) was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

a.



b.

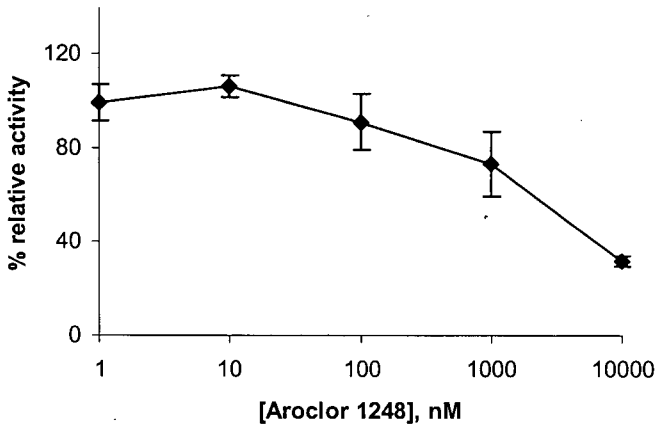


Figure 8. LNCaP cells transfected with AR and exposed to a) Aroclor 1248 + 0.05 nM DHT b) Aroclor 1248 + 1 nM DHT. Concentration of Aroclor 1248 in nM (a)+ 0.05 nM DHT b) + 1 nM DHT) is plotted on the x axis, and the percent fold activation with the control activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and control activity (activity before DHT added) was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

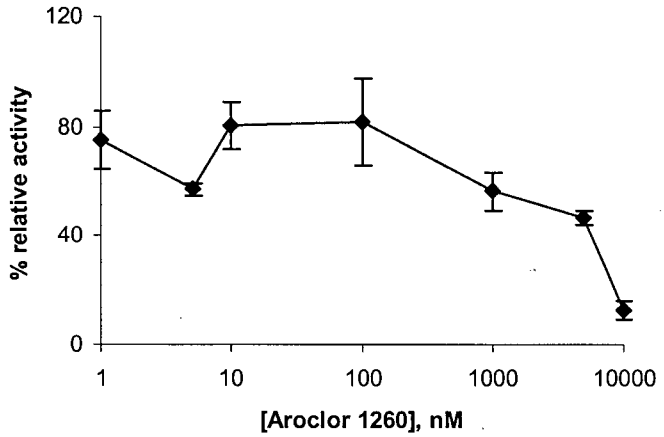
3.1.4 Alteration of Luciferase Activity Induction Through AR by Aroclor 1248

Aroclor 1248 did not have a significant effect on luciferase activity with AR in the absence of hormone. In the presence of a half maximal level of DHT, there was no change in luciferase activity at 1 or 10 nM, but a strong antagonistic effect above 10 nM Aroclor 1248 was seen with a distinct decrease in activity at 100 nM (Figure 8a). This was followed by a further shallow decrease in activity from 1000 to 10000 nM Aroclor 1248 reaching a 99% decline in luciferase activity. A less striking effect was seen when a saturating level of DHT was used (Figure 8b). In this case, there was no decrease in activity from 1 to 10 nM. However, a slight decrease in luciferase activity was seen at 10, 100, and 1000 nM and a sharp decrease at 10000 nM at which point the luciferase activity had diminished by 69%. A change in luciferase activity was not observed with GR in the absence of hormone or the presence of DEX.

3.1.5 Alteration of Luciferase Activity Induction Through AR by Aroclor 1260

Aroclor 1260 did not have a marked effect on luciferase activity with AR in the absence of hormone. In the presence of 0.05 nM DHT there was an antagonistic effect shown by a slight decrease in activity at 1000 nM and a sharp decrease at 10000 nM by 88% (Figure 9a). In the presence of saturating levels of DHT, Aroclor 1260 does not cause a distinct change in luciferase activity at 1 nM or 10 nM. Aroclor 1260 was a strong antagonist above 10 nM Aroclor 1260, as shown by an abrupt decline in luciferase activity at 100 nM that remains reduced by approximately 60% for all higher

a.



b.

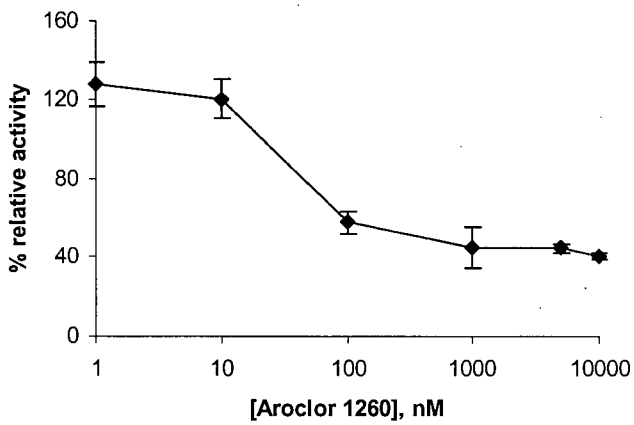


Figure 9. LNCaP cells transfected with AR and exposed to a) Aroclor 1260 + 0.05 nM DHT b) Aroclor 1260 + 1 nM DHT. Concentration of Aroclor 1260 in nM (a)+ 0.05 nM DHT b) + 1 nM DHT) is plotted on the x axis, and the percent fold activation with the control activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and control activity (activity before DHT added) was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

concentrations (Figure 9b). There was not a discernible alteration of luciferase activity with GR in the absence of hormone or the presence of DEX.

3.1.6 Alteration of Luciferase Activity Induction Through AR and GR by PCB 42

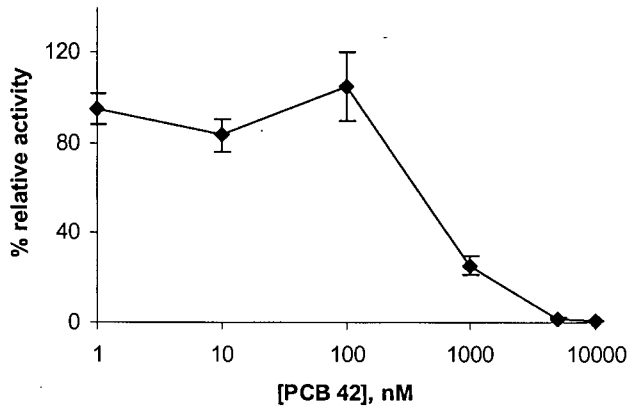
PCB 42 did not have a detectable effect on luciferase activity with AR in the absence of hormone. PCB 42 had a profound antagonsitic effect with AR in the presence of 0.05 nM DHT as shown by a marked decrease in luciferase activity at 1000 nM PCB 42 (Figure 10a). This is followed by a less steep but certainly notable decrease in activity by approximately 98%. In the presence of 1 nM DHT there is no distinct change in activity observed from 1 to 1000 nM. However there is a very abrupt decrease in activity by 93% at 5000 and 10000 nM PCB 42 (Figure 10b).

Using GR, in the absence of DEX there was no effect on luciferase activity. However, when 1 nM DEX was added to the cells, PCB 42 caused a sharp increase in luciferase activity from 1 to 100 nM, followed by a leveling off of luciferase activity at higher concentrations with a 150% increase (Figure 10c).

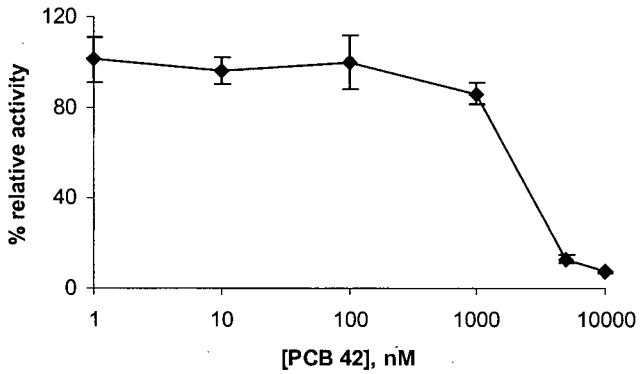
3.1.7 Alteration of Luciferase Activity Induction Through AR by PCB 31

PCB 31 had an antagonistic effect on luciferase activity with AR in the presence of 0.05 nM DHT. There was no change in activity from to 100 nM, however, there was a moderately distinct decrease by 60% in luciferase activity at higher concentrations (Figure 11). There was no effect on luciferase activity when 1nM DHT or DEX was used.

a.



b.



c.

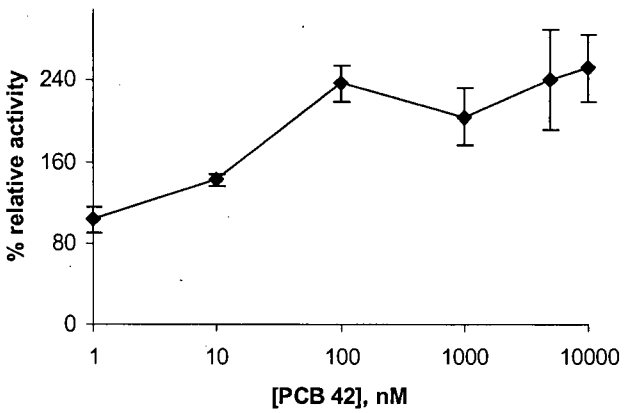


Figure 10. LNCaP cells transfected with AR and exposed to a) PCB 42 + 0.05 nM DHT b) PCB42 + 1 nM DHT, and transfected with GR and exposed to c) PCB 42 + 1 nM DEX. Concentration of PCB 42 in nM (a)+ 0.05 nM DHT b) + 1 nM DHT) c) + 1 nM DEX is plotted on the x axis, and the percent fold activation with the control activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and control activity (activity before hormone added) was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

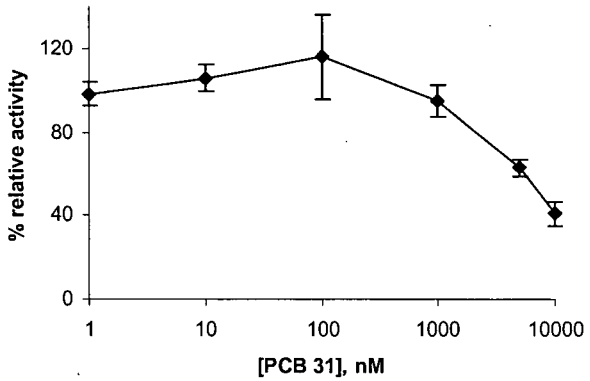


Figure 11. LNCaP cells transfected with AR and exposed to PCB 31 + 0.05 nM DHT. Concentration of PCB 31 in nM + 0.05 nM DHT is plotted on the x axis, and the percent fold activation with the control activation level set at 100% plotted on the y axis. Luciferase activity was normalized relative to Renilla luciferase activity, and control activity (activity before DHT added) was set at 100% fold activation (not shown on log graph). Data expressed as the mean fold activation ± SEM.

3.2 Displacement of ^{3}H-DHT as AR Ligand by PCBs

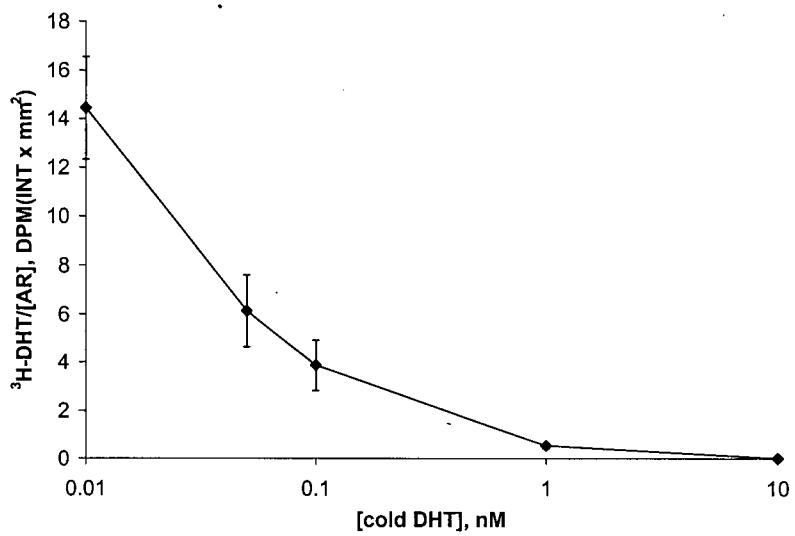
There are a number of mechanisms by which xenobiotics can alter steroid hormone action. One of these well established mechanisms is through modification of steroid hormone binding to the receptor. In order to determine if the androgen receptor is a target for PCB action, a cell culture assay using a HeLa cell line with a stably transfected FLAG-tagged AR was employed (*140*). Cells were exposed to ^{3}H-DHT (specific activity =123 Cii/mmol), a known ligand of AR, in combination with an excess of each of the PCBs tested in the luciferase reporter gene assay. Once the AR binds to ligand, this complex translocates to the nucleus. Therefore, extraction of the nuclear fraction of these cells shows the location of the ^{3}H-DHT, and demonstrates the ability of the Aroclors and individual congeners to interfere with the some of the processes involved in androgen action, including ligand binding to the AR. These experiments were designed to provide insight into the complex mechanisms of PCB interference with steroid hormones, which has not been previously extensively examined at the cellular or molecular level in this manner.

Figure 12 depicts the level of 3H-DHT binding when a) unlabelled DHT is added as competitor, and b) Aroclor 1254, Aroclor 1242, Aroclor 1248, Aroclor 1260, PCB 42, and PCB 31 are competitors of 0.3 nM ^{3}H-DHT.

There was a profound decrease in bound ^{3}H-DHT at 10000 nM for all PCBs except for PCB 31, which did not affect the amount of ^{3}H-DHT bound to the AR. A 50% decrease was observed at 1000 nM with Aroclor 1242 and PCB 42, and a 25% decrease at 1000 nM with Aroclor 1248, 1254 and 1260. At this concentration, only PCB 42 caused a significant ($p<0.05$) reduction in bound ^{3}H-DHT. At 10000 nM, a 90-95% decrease in bound DHT was observed for Aroclor 1242, Aroclor 1254, and PCB 42, and

a 60% decrease for Aroclor 1248 and Aroclor 1260. PCB 42 and Aroclor 1254 reduced the bound ^{3}H-DHT significantly ($p<0.05$).

a.



b.

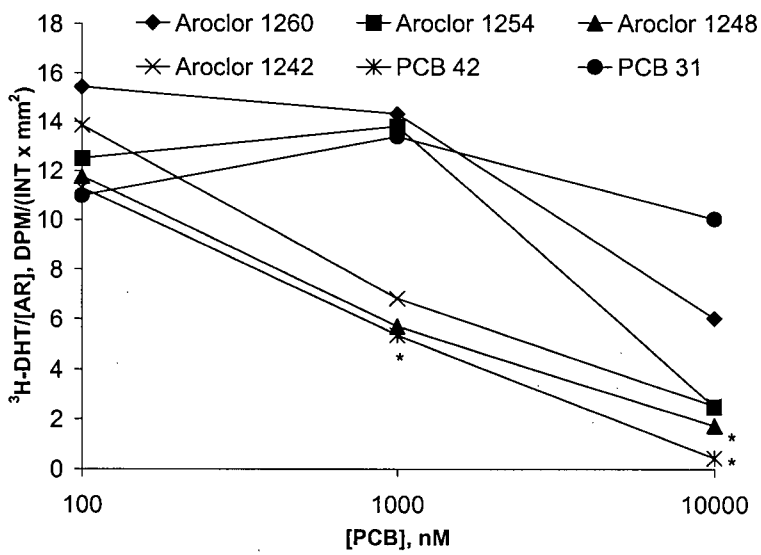


Figure 12. HeLa FLAG-tagged AR cells exposed to a) DHT titration + 0.3 nM ^{3}H-DHT and b) PCB titrations + 0.3 nM 3H-DHT. Bound ^{3}H-DHT normalized relative to AR concentration based on quantitative analysis of Western blotting, and control activity was set at 100% bound (not shown on log graph). Data expressed as the mean bound ^{3}H-DHT. * indicates statistical significance ($p < 0.05$).

3.3 Analysis of CAT Activity

The *in vitro* cell culture studies demonstrated that PCBs have properties of potential endocrine disruptors. More specifically, the PCB mixture Aroclor 1254 was able to induce reporter gene activity through the AR in the absence of known ligand and also reduced reporter gene activity in the presence of the ligand DHT. This novel finding of an alteration in AR-driven activity was particularly notable above 100 nM Aroclor 1254 in the presence of half maximal levels of DHT. In addition, the ligand displacement assay showed that 10000 nM Aroclor 1254 prevents 90% of ^{3}H-DHT from being associated with the AR. These results, as well as reports of the ability of Aroclors to alter steroid hormone systems and reproductive development, warranted an examination of *in vivo* effects of Aroclor 1254, particularly on the prostate, a sensitive target organ of androgens.

In order to measure the influence of Aroclor 1254 on prostate development, the expression of the androgen-driven prostate specific LPB-CAT transgene and the effects on mouse organ weights were investigated. The organs examined were androgen-target and reproduction related organs such as testes, epididymis, and prostate; as well as liver, an established target for PCB effects as well as the site of steroid hormone metabolism.

PCBs are known to bioaccumulate and are stored preferentially in adipose tissue, but are also present in serum, blood plasma, and milk (*145*). Consequently, PCBs can be transferred to offspring via the placenta and through breast milk (*48, 146*). Therefore, the offspring of exposed mothers, who are exposed to PCBs at early and sensitive stages of life can be more susceptible to the effects of PCBs than those exposed during adulthood.

Two studies were undertaken to evaluate the effects of Aroclor 1254 exposure during development. These studies were designed to look at chronic exposure to PCBs through different stages of development, rather than acute exposure which is tested in many toxicology studies. In the single dose level study, dams were dosed with 10 mg/kg/day from the time of mating, until the end of the lactation period at 3 weeks. Weanlings were then administered 10 mg/kg/day Aroclor 1254 until four weeks of age (prepubertal) and eight weeks of age (post pubertal), in order to assess the effects of Aroclor 1254 at different stages of development. In the dose response study, dams were treated with 10, 20, 40 mg/kg/day Aroclor 1254 from the time of mating, until the end of the lactation period at 3 weeks. Weanlings were then administered the corresponding dose until 8 weeks of age, to test the effects of different doses of Aroclor 1254 on the prostate.

3.3.1 Single dose level Group

There was a reduction in CAT activity from the prostate extracts of the four and eight week treatment mice, which was statistically significant ($p>0.05$) in the eight week group (Figure 13). These findings show antiandrogenic action of the Aroclor 1254 treatment on the specifically androgen responsive LPB-CAT reporter. Due to this effect at a relatively low dose of 10 mg/kg/day, we chose to look at mice at eight weeks of age, treated from conception in the same manner, but also using treatments 2 and 4 fold higher.

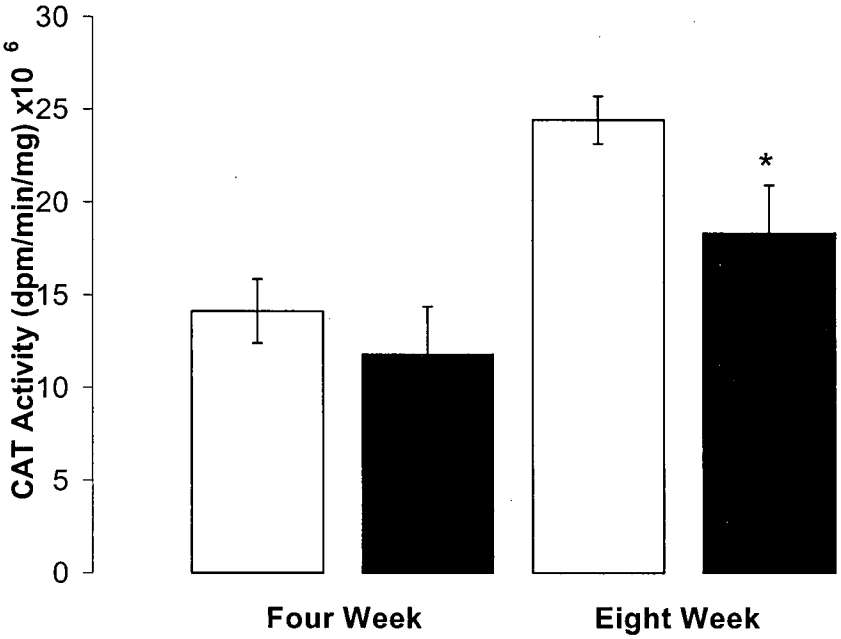


Figure 13. Activity of CAT reporter transgene linked to the prostate specific probasin promoter from prostate tissue extracts of four and eight week old control (open bars) and treatment (closed bars) LPB-CAT mice in the single dose level study. Mice were treated with 10 mg/kg/day Aroclor 1254 by gavage from conception until four or eight weeks of age. Data is presented as mean CAT activity. Error bars represent SEM. *$p<0.05$ (Student's *t*-test).

3.3.2 Dose Response Group

The CAT enzyme activities in the dose response group did not show any significant difference between groups ($p<0.05$), despite the trend of a decrease in activity at higher doses. The activities of the prostate extracts from the control group were also remarkably lower than those in the single dose level study, and were lower than the 10, 20, or 40 mg/kg/day group CAT activities (Figure 14).

3.4 Alteration of Organ Weights

In order to assess the effects of PCBs on the mice used in this study, changes in body weight and organ weight were useful parameters that were employed. Body weights of mothers being dosed, suckling pups, and weanlings were collected throughout both studies. Due to litter to litter differences, the treatment mice in the single dose level group were heavier than the control group. An increase in body weight is not characteristic of PCB exposure, and in fact, a decrease in body weight has been reported as a health effect of these compounds (*59*). The differences in body weight in the mice in this study may be attributable to litter size. Some of the treatment mice were from smaller litters, which can yield larger pups. However, there was no trend in litter size observed when treatment and control groups were compared. This pattern was not seen in the dose response group, where there were no measurable differences seen between dose groups. Organ weight was corrected for individual body weight, due to the variation in body weight between individual animals in this study.

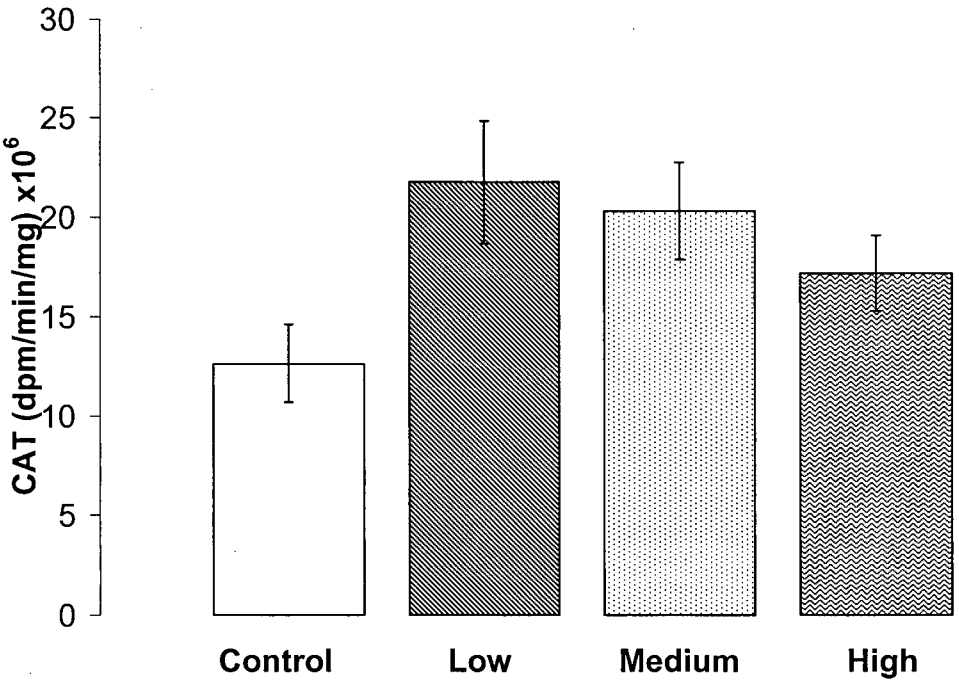


Figure 14. Activity of CAT reporter transgene linked to the prostate specific probasin promoter from prostate tissue extracts of eight week old LPB-CAT mice in the dose response study treated with vehicle only (control), 10, 20, and 40 mg/kg/day. Mice were treated with 10, 20, 40 mg/kg/day Aroclor 1254 by gavage from conception until eight weeks of age. Data is presented as mean CAT activity. Error bars represent SEM.

3.4.1 Liver

To determine if there was an effect on the liver, a known target organ of PCBs, we examined the livers of treated mice for changes in weight, although the doses used were below levels shown to cause overt effects in other studies (*144*). There were no significant differences in liver weight/body weight between control and treatment mice in the single dose level study (Table 2). Liver weight/body weight in the dose response treatment mice were significantly higher for the 20 and 40 mg/kg/day dose groups than controls (Table 3). This is consistent with reports in the literature of PCB liver effects (*44, 73, 74, 76-78*). Increase in liver weights due to PCB exposure is usually associated with hepatocyte enlargement, which is not considered an adverse effect, unless certain biochemical changes along with notable histological alterations are also observed (*59*).

3.4.2 Prostate

The prostates in the single dose level group did not show a significant difference in prostate weight to body weight ratio between treatment and controls for the single dose level group (Table 2). In the dose response study, prostate weight/body weight was altered by Aroclor 1254 treatment. Prostate weight/body weight for the 20 and 40 mg/kg/day dose groups were significantly lower than the control group (Table 3).

		CONTROL (N=12)	TREATMENT (N=5)
	Prostate	2.99±0.20	2.7±0.25
	Epididymis	7.69±0.26	7.2±0.22
Four Weeks	Testis	27.15±1.38	28.4±0.70
	Heart	51.84±0.94	49.16±3.3
	Liver	537.42±5.25	625.66±11.49**
	Kidney	69.12±1.76	65.16±1.98

		CONTROL (N=11)	TREATMENT (N=8)
	Prostate	4.38±0.27	3.99±0.29
	Epididymis	13.85±0.28	13.16±0.70
Eight Weeks	Testis	42.17±2.12	43.87±1.81
	Heart	50.58±1.40	46.61±2.14
	Liver	494.10±15.00	540.05±21.88
	Kidney	72.38±2.25	72.86±3.21

*p<0.05, **p<0.01(Student's *t*-test)

Table 2. Corrected organ weights for prostate, left epdidymis, left testis, heart, liver, and kidney (organ weight/body weight in grams/gram body weight $x10^4$) of LPB-CAT mice treated to four and eight weeks of age with vehicle only (canola oil) and 10 mg/kg/day Aroclor 1254. Values are mean ± SEM.

	CONTROL (N=9)	10 MG/KG/DAY (N=5)	20 MG/KG/DAY (N=11)	40 MG/KG/DAY (N=5)
Prostate	4.36±0.25	3.91±0.23	3.50±0.18*	3.20±0.27**
Epididymis	13.63±0.21	12.41±0.62	13.33±0.39	13.03±0.45
Testis	46.92±2.22	41.71±3.79	48.79±1.09	47.86±1.11
Heart	51.30±1.27	47.21±0.80	49.05±0.38	51.48±2.41
Liver	496.53±9.67	536.39±14.54	617.58±13.52**	718.08±24.26**
Kidney	74.72±2.44	75.63±2.59	75.67±1.70	78.30±2.72

*p<0.05 ,**p<0.01(analysis of variance and Dunnett's test)

Table 3. Corrected (organ weight/body weight in grams/gram body weight $x10^4$) for mice treated for eight weeks of age with canola oil, Aroclor 1254 at 10, 20, 40 mg/kg/day. Values are mean ± SEM.

3.4.3 Serum Testosterone Levels

The level of testosterone in the serum may provide insight into the effects of antiandrogens on the endocrine system. This has been seen in studies of antiandrogen drugs used in prostate cancer treatment (*147-149*), as well as in the treatment of aggression in convicted male sexual offenders (*132*). Patients treated with the antiandrogen flutamide, develop an increase in serum testosterone levels due to the inhibition of the negative feedback effects of androgens on the hypothalamic-pituitary-testes axis (*150*). Serum lutenizing hormone (LH) is also increased in a dose related manner with flutamide administration (*151*), and it is through negative feedback control by endogenous androgens of gonadotropin secretion in the hypothalamus that LH secretion is regulated (*152*).

There was a high degree of variation in testosterone levels within treatment groups. However, this is consistent with other studies in which the measured serum testosterone levels ranged from 0.7 nmol/l to 158 nmol/l in post pubertal mice (*153*) (M. Bowden, personal communication). Variations in serum testosterone levels in mice may be attributed to the release of LH from the pituitary in pulses (*154*). As testosterone synthesis is dependent on LH, these fluctuations may be expected and data was therefore presented as a scatter plot of testosterone concentrations (Figure 15). There were three serum testosterone levels that were much higher than the other fifteen samples measured. Statistical analysis of the data set was done both with and without these three points which may have skewed the results. ANOVA tests of the data with and without the higher testosterone levels both showed no significant difference between treatment groups ($p<0.05$). The Kruskal-Wallis test was also used, as this test ranks the data and

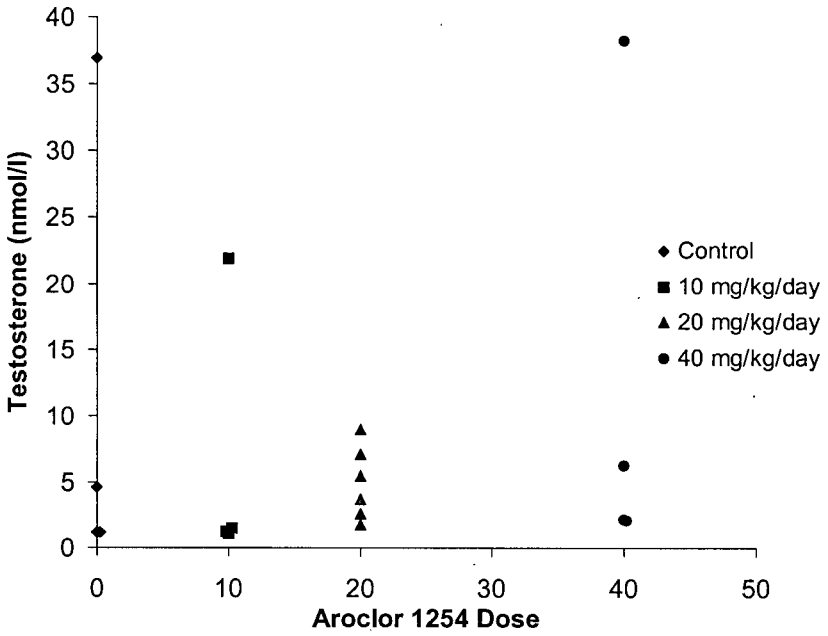


Figure 15. Scatter plot of serum testosterone levels from eight week old LPB-CAT mice in the dose response study treated with vehicle only (control), 10, 20, 40 mg/kg/day.

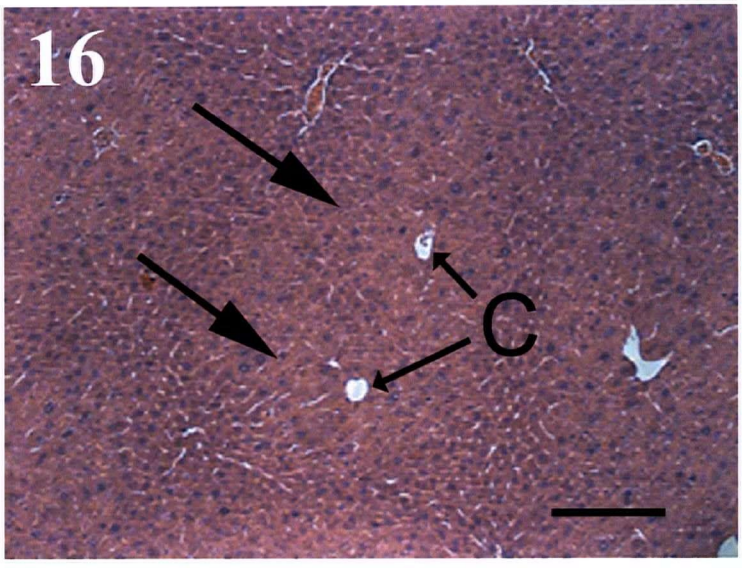
uses the median of each group, rather than the mean and thus a valid result could be obtained and the inclusion of the three high testosterone levels would not be problematic to analysis. This test also found no statistical difference between treatment groups ($p<0.05$).

Compromised liver function is a possibility when agents with known hepatic effects are used. Impaired hepatic function or inflammation with an increase in liver enzymes may reduce the liver's ability to remove aromatized testosterone metabolites from the serum. These metabolites are weakly estrogenic and reduce testosterone levels by negative feedback on the pituitary. Although a wide range of testosterone levels was observed, the testosterone levels are within a normal range (*153*). The results from the testosterone assay are not conclusive regarding the effects of Aroclor 1254 on liver function and hormone metabolizing capacity; however, this does not negate the other notable findings in this set of studies.

3.5 Pathological Analysis of Histological Sections

3.5.1 Liver

Histological analysis of livers of mice from each treatment group in the dose response study were performed to confirm that the alteration in liver weight was not a result of severe toxicological changes that might affect liver function. The livers of Aroclor 1254 treated mice showed changes in the distribution of hepatocellular vacuolization. Control and 10 mg/kg/day treated mice showed no vacuolization of hepatocytes around the centrilobular veins, whereas 9% of the medium dose mice and 80% of the high dose mice livers had centrilobular vacuolization (Figure 16). Control mice had diffuse or no obvious regional distribution of vacuoles in 89% (8 of 9) of the cases examined. Low dose mice had 40%(2 of 5), medium dose mice had 9% (1 of 11),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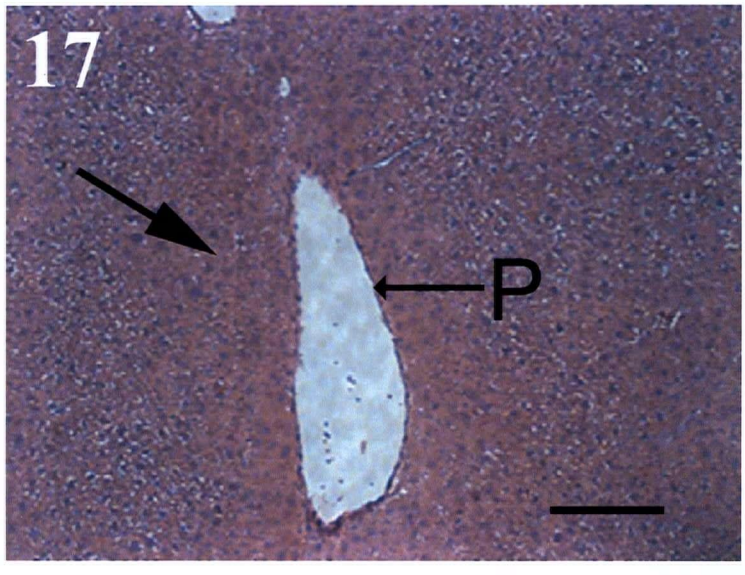
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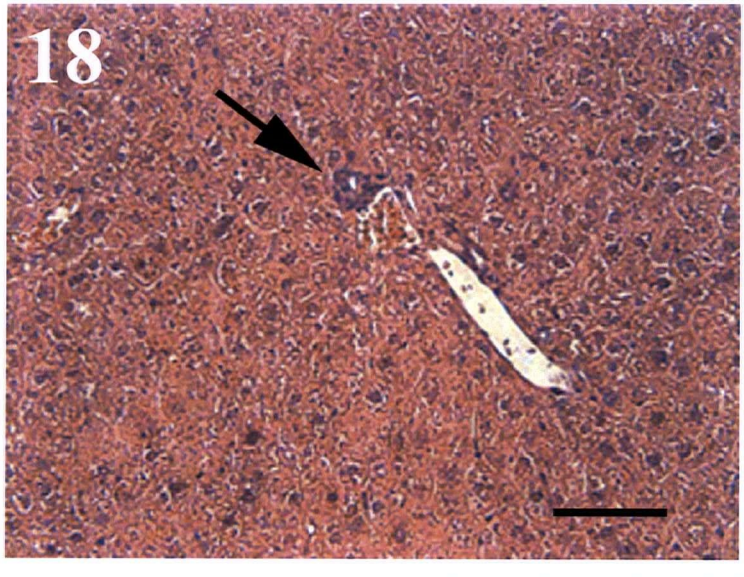
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Figure 16. Centrilobular vacuolization in the liver of an eight week old mouse treated with 40 mg/kg/day of Aroclor 1254. The hepatocytes surrounding the centrilobular veins (C) are highly vacuolated (arrows) compared to cells in other regions of the liver. This vacuolization is associated with increased intracytoplasmic glycogen accumulation. This histological finding was only present in mice dosed with 20 and 40 mg/kg/day. H & E staining. Bar= 10μM.

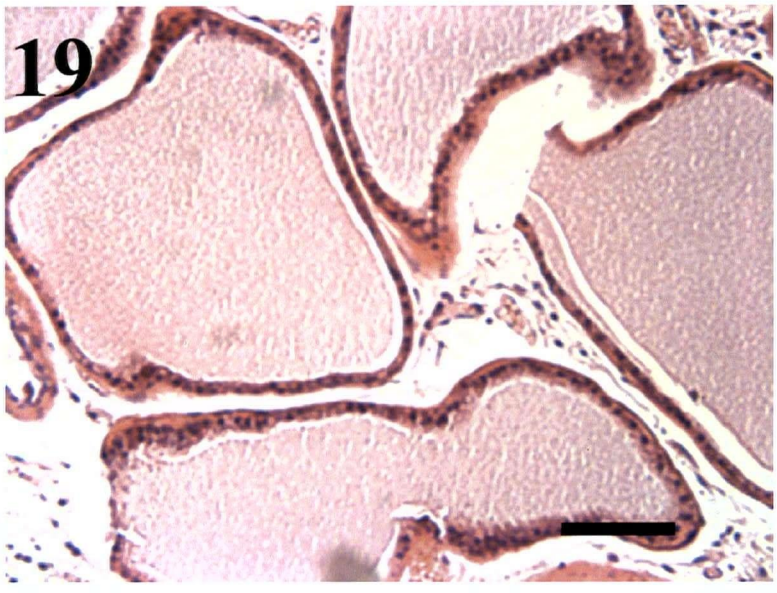
Figure 17. Periportal vacuolization in the liver of an eight week old mouse treated with 20 mg/kg/day of Aroclor 1254. There is a high degree of vacuolization in the liver cells surrounding the periportal vein (P) relative to other areas of the liver (arrow). This type of vacuolization was observed in all treatment groups, and was most common in the liver of mice treated with 10 and 20 mg/kg/day. H & E. Bar= 10μM.

Figure 18. Accumulation of lymphocytes and small macrophages in periportal spaces (arrow) in the liver of an eight week old mouse treated with 20 mg/kg/day. The lesions that were observed were mild, although they were more common and more severe in the mice treated with 20 and 40 mg/kg/day. H & E. Bar= 10μM.

and high dose mice had no cases in which there was diffuse vacuole distribution. There was also vacuolization around the periportal areas (Figure 17). This was seen in 11% (1 of 9) of the control mice livers, 60% (3 of 5) in the low dose, 82% (9 of 11) in the medium dose, and 20% (1 of 5) in the high dose. There was also an increase of infiltration of lymphocytes and small macrophages in the periportal spaces of the livers of treated mice. This was observed in 33% (3 of 9) of control mice, 20% (1 of 5) of low dose mice, 72% (8 of 11) of medium dose mice, and 100% (5of 5) of high dose mice (Figure 18). Although this type of change is often considered incidental in mice, the change was more common and more severe (based on pathologist's grading) in the medium and high dose groups which suggests an association with Aroclor 1254 treatment. This finding is consistent with that in the literature (*44, 73, 74, 76-78*). The cellular changes were mild and were not considered clinically significant (causing disease) by the pathologist.

3.5.2 Prostate

Histologically, there was a change in the predominant structure present in the prostates of treated mice in the dose response study. There was an increase in the percentage of cases in which dilated acini was the predominant structure, as dose increased. Dilated acini which were considered acini that were filled with proteinaceous material, were lined with a single row of cuboidal epithelial cells with some intraluminal infolding. A single layer of myoepithelial cells surrounded these acini. Dilated acini (Figure 19) was the predominant structure in 44% (4 of 9) of control prostates, 60% (3 of 5)of low dose, 73% (8 of 11) of medium dose, and 80% (4 of 5) of high dose. In addition, there was a dose-related decrease in the percentage of cases in which non-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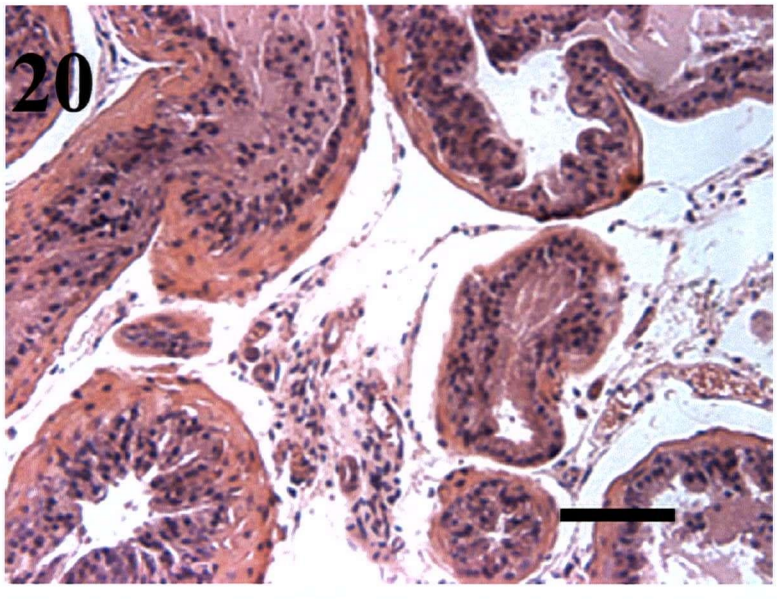
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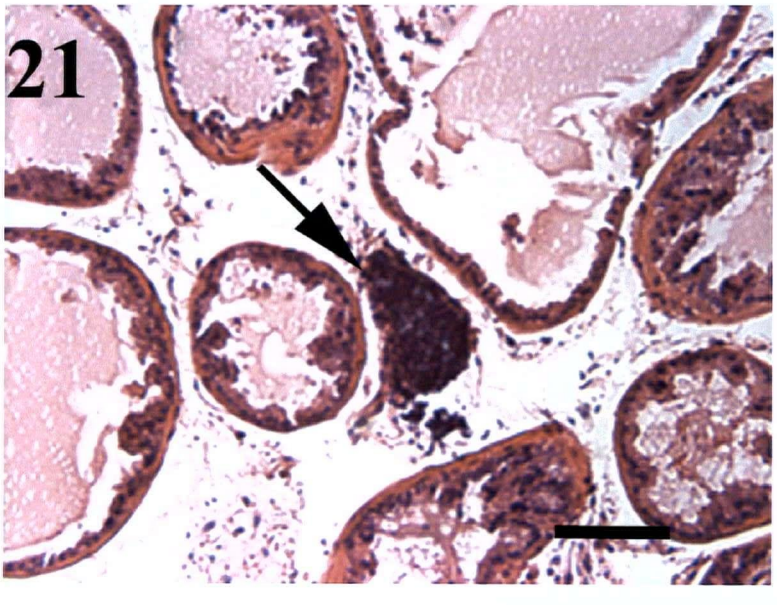
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Figure 19. Dilated acini of the prostate of an eight week old mouse control group mouse. This was found to be the predominant structure in the mouse prostates in an Aroclor 1254 dose related manner. H & E. Bar= 5μM.

Figure 20. Non-dilated acini of the prostate of an eight week old control group mouse. This was found to be the predominant structure in the mouse prostates in an inverse Aroclor 1254 dose related manner. H & E. Bar= 5μM.

Figure 21. Infiltration of discrete aggregates of small lymphocytes (arrow) in the interstitial tissue of the prostate of an eight week old mouse treated with 40 mg/kg/day of Aroclor 1254. These infiltrates were present in the prostates of treatment mice but were not detected in the prostates of control mice. H & E. Bar= μM.

dilated acini was the predominant structure. Non-dilated acini (Figure 20) were smaller and contained less proteinaceous material than the dilated acini. They were lined by one to several rows of densely packed, low columnar to columnar, frequently disorganized, epithelial cells. There was a greater degree of infolding than in the dilated acini. One to three layers of myoepithelial cells surrounded the non-dilated acini. This structure was predominant in 56% (5 of 9) of controls, 40% (2 of 5) of low dose, 27% (3 of 11) of medium dose, and 0% (0 of 5) of high dose prostatic tissue examined.

Histological analysis of the prostates also showed intravascular multifocal lymphocytic infiltration in the prostatic stroma of mice treated with Aroclor 1254 (Figure 21), while no such infiltration was seen in the prostates of control mice. This infiltration consists of random and multifocal infiltration of the interstitial tissue by discrete aggregates of small lymphocytes with undistinguishable cytoplasm. These aggregates were often in the lumen of small vessels. There was no obvious change in periductular focal lymphocytic infiltration in the prostatic stroma of the different groups. This was characterized by the presence of well-differentiated lymphocytic aggregates with scarce but distinguishable cytoplasm in the myoepithelial layers of the prostatic ductules. None of the lymphocytic infiltrates were associated with any detectable histological alterations of the glandular component of the prostate. Despite the lack of structural changes, the cause of the presence of the infiltrates has yet to be demonstrated.

3.5.2.1 Comparison of Stromal Areas in Prostate Tissue

The dose related increase in dilated acini in combination with the dose related decrease in prostate weight suggested that this may indicate a loss of a specific structure type in the prostate due to Aroclor 1254 treatment. Therefore, the stromal and ductal

areas in the prostate tissue slides were measured using Adobe Photoshop 5.5. The number of pixels for each structure was counted in 3 random images of the stained prostate slide for each mouse. There was no correlation between the treatment, and the percent stromal area (Figure 22).

3.5.3 Testes

Testes of mice from the different dose groups were also examined and found to be histologically normal is all cases. The tissue consisted of numerous well-differentiated seminiferous tubules separated by the septula testis. The stroma contained a normal number of Leydig cells. Active spermatogenesis was found in all tubules, as characterized by the presence of numerous spermatogenic cells.

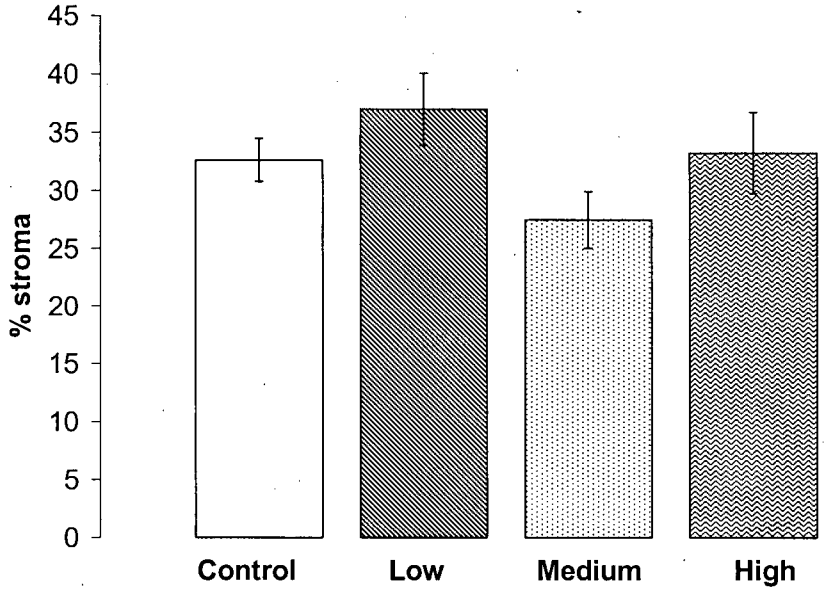

Figure 22. Percent ratio of stromal area to total area in prostate tissue from mice in the dose response study.

CHAPTER 4.

DISCUSSION

The presence of xenobiotic chemicals in the environment has raised concerns in the scientific community regarding the endocrine modulating effects of these compounds in wildlife and humans and the detrimental effects that arise in fertility, sexual development, and reproductive tract cancers (*9*). The ability of environmental contaminants to alter the development and function of hormone target organs such as the prostate has shown the importance of testing chemicals in the environment for their endocrine disrupting potential. The role of PCBs and PCB metabolites as estrogens has been previously documented (*86*). However, the effects of PCBs (and other environmental chemicals) on androgen mechanisms has not been thoroughly investigated and has not been pursued by many investigators in this field of study. Bioassays using reporter genes, ligand displacement, and an androgen regulated prostate specific transgene were used in this study to gain new information pertaining to the capacity of PCBs to influence steroid hormone action through the androgen axis *in vitro* and *in vivo*.

4.1 Luciferase Reporter Gene Assay

The ability of PCBs to alter DHT induced luciferase activity in the reporter gene system through the AR was tested at half maximal and saturation levels of DHT, as well as in the absence of DHT. This study showed that Aroclor 1254 can induce an activity that is similar to androgenic activity in prostate cells in culture in the absence of a natural AR ligand. In addition, in the presence of half-maximal DHT, Aroclor 1254, Aroclor

1242, Aroclor 1248, Aroclor 1260, PCB 31, and PCB 42 were antagonistic and reduced the activity of the androgen driven luciferase reporter gene.

When saturating levels of DHT were used in combination with the PCBs tested, similar trends were seen. All the Aroclor mixtures tested and PCB 42 showed a reduction in luciferase activity at high concentrations when 1 nM DHT was present. The trend of these curves tended to be less steep compared to experiments in which the transfected cells were exposed to 0.05 nM DHT. This may be because when cells are exposed to saturating levels of DHT there is more DHT for the PCB molecules to compete with. However, PCB 31 did not show any change in luciferase activity when saturating levels of DHT were added to the cell culture medium.

Similar experiments testing the effects of these PCB mixtures and congeners through the GR showed that one PCB, the individual congener 42 was able to induce transcription in the presence of half maximal DEX. PCB 42 did not interact with the GR to drive the reporter construct in the absence of DEX. This result may be due to altered cellular distribution of the steroid hormones. Changes in the import and export of steroid hormones such as glucocorticoids due to the presence of PCB 42 could cause the increase in reporter activity that is observed, in that an inhibition of steroid hormone export from the cell would increase cellular levels of these hormones. It is also possible that this congener is binding to serum binding proteins (SBPs) and causing an increase in the amount of DEX that is in the cell, as binding sites on the SBPs are occupied by PCB 42. This mechanism has been suggested for other endocrine disruptors influencing steroid hormone activity in the cell (*15*). Here the investigators found that δ-hexachlorocylohexane, methoxychlor, p,p'-DDT, p,p'-DDE, and atrazine all reduced the

binding of ^{3}H-5α-DHT to the ABP of rat epididymal cytosol by a statistically significant level. In addition, δ and γ hexachlorocyclohexane, o',p'-DDT, pentachlorophenol, and nonylphenol all significantly reduced the binding of ^{3}H-5α-DHT to sex hormone binding globulin in human postpartum serum. By reducing the amount of steroid hormone bound to the binding proteins, changes in signal transduction can occur due to the increase in hormone that is free to enter the cell. Finally, PCB 42 may inhibit the cytochrome P450 enzymes needed to metabolize DEX, thereby resulting in an increase in DEX in the cell and higher levels of reporter protein activity, as has been seen with some P450 enzymes responsible for steroid hormone metabolism (*155, 156*). PCB 42 was the only compound in this study that induced transcription through the GR. This suggests that the other PCB compounds have activity *in vitro* that is specific to the AR in comparison to GR.

4.2 Ligand Displacement Assay

All the Aroclor mixtures tested, as well as PCB 42, demonstrated an ability to reduce ^{3}H-DHT bound to the AR when the amount of labeled ligand bound to the FLAG-tagged AR from a stably transfected HeLa cell line was measured. However, PCB 31 did not alter ^{3}H-DHT-AR binding. This finding, in combination with the lack of luciferase reporter gene activity in the presence of saturating levels of DHT suggests that PCB 31 does not have the same abilities as the other PCBs tested to act via the AR. It is also possible that this compound does have weak antiandrogenic properties and that the binding capacity was not detected in the test system used in this study.

The ligand displacement assay results indicate that these PCBs can reduce natural ligand binding. The mechanisms that produce such changes are not clear. It is possible that PCBs bind directly to the AR and prevent the natural ligand from binding. Some hydroxylated PCB metabolites have been shown to bind to ARs in the kelp bass and

atlantic croaker causing 50% displacement of ^{3}H-testosterone (*157*). Alternatively, PCBs may block DHT import into the cell or cause an increase of the rate that DHT is exported from the cell.

There are certain limitations to working with complex industrial mixtures such as Aroclors, in that it is not clear which components are responsible for the results seen. The induction of transcription as well as the prevention of AR ligand binding may be due to action of certain congeners on their own, or a combined effect of many components of the Aroclor mixtures. It is also possible that the PCBs tested in both the *in vitro* experiments were metabolized by the cells in culture. PCB metabolites, particularly hydroxylated PCBs may be responsible for some of the changes in luciferase reporter induction, as well as ^{3}H-DHT association with AR.

4.3 CAT Assay

The single dose level study of CAT enzyme activity in the prostate showed a significant decrease in activity at 8 weeks of age. This is the first time that PCBs have been shown to be antiandrogenic in a sensitive animal model system using an androgen regulated reporter gene.

While there was a decrease in mean CAT activity in the prostates of the 4 week old mice, this was not significantly different than the control group.

The dose response study showed a non significant dose related decrease in CAT activity from 10 to 40 mg/kg/day. In this study, the control group CAT activity was significantly lower than the treatment groups. The source of this reduction may be in tissue preparation. Alternatively, the vehicle used in this study, canola oil, may contribute to the decrease in CAT activity. The canola oil for the dose response group was obtained from a different source than that used for the single dose level group.

Although no effects were seen in the other parameters measured, it is possible that contaminating components of the oil caused a decrease in CAT activity in the dose response group. Canola oil is similar to vehicles used in studies of organochlorine toxicity and reproductive effects (*44, 101, 114, 133*) and is a normal dietary component. The decrease in CAT activity is not observed in the groups treated with canola oil containing Aroclor 1254 and significant effects on CAT activity and reproductive function were not observed in other studies using canola oil in our laboratory. Vehicle free controls may have added additional data to this study, however, this control was not considered as part of the initial experimental design. In addition, the levels of CAT activity seen in the control group of the dose response study is similar to the values reported by other investigators performing a similar study of mice treated with an identical protocol using a different source of canola oil (Dr. C. Butler, unpublished results). The CAT activity levels from the 8 week mice in the single dose level study were approximately 2 fold higher than in the dose response study. This is consistent with variability reported using this model in our laboratory, and in the literature (*143*). This suggests that extreme precautions are required in tissue preparation and analysis of CAT enzyme activity.

4.4 Organ Weights and Histopathology

4.4.1 Liver

The dose related increase in liver weight in mice exposed until 8 weeks of age to 20 and 40 mg/kg/day Aroclor 1254 is similar to liver effects reported in other studies (*44, 73-78*). Histological changes were also observed in the livers of mice treated with higher doses. These dose related alterations, such as increased vacuolization due to glycogen

accumulation (*158*) and lymphocytic infiltrates in perportal spaces, are considered pathologically mild and are not associated with organ dysfunction.

The serum testosterone levels are within a normal range for all dose levels. Therefore, although mild changes were observed in the liver, the results do not suggest that liver function, such as the ability to metabolize testosterone, is significantly compromised by Aroclor 1254 treatment in this study.

Studies of changes in the activity of CYP enzymes involved in steroid hormone metabolism due to PCB exposure has been shown by other investigators (*159-162*). One of these studies found a significant decrease in CYP 2c and in serum testosterone levels with exposure to 3,4,5,3',4',5'-hexachlorobiphenyl (*161*). However there was not a causal relationship between these two physiological changes (*162*), and alteration in testicular androgenesis has been suggested (*160, 162*).

4.4.2 Prostate

A dose related decrease in prostate weight was observed in the dose response group. These findings are consistent with other studies of rodents treated with PCBs (*114*). This trend may be due to apoptosis, as is seen after castration, in which the lack of androgens produces rapid involution and apoptosis in the prostate. Another possibility is an alteration in prostate growth throughout development due to the influence of PCBs.

The histological findings in the prostate showed that dilated acini were the predominant structure in the ventral prostates in a dose related manner. Similar results were found in a previous study, in which male rats treated with 8, 32, and 64 mg/kg of Aroclor 1254 during lactation developed fewer acini, less folding of the mucosa, and flattened epithelial cells in the acini of the ventral prostate (*114*). These observations

were more pronounced in the rats exposed to the highest dose of Aroclor 1254. The observation of fewer acini may be due to the dilation of the acini and the presence of prostatic fluid within. A lesser degree of infolding of the mucosa would also be consistent with the dilation of the acini, which was observed by the pathologist in this study. It is not clear if the presence of flattened rather than cuboidal epithelial cells is an indication of a group of cells that are less active or simply flattened due to the pressure of secretions that are filling the acini. Due to the function of this structure, the latter is a valid possibility.

Lymphocytic infiltrates in the prostatic stroma of treated mice in a dose related manner was considered an abnormal histological finding. The presence of these cells correlates with a decrease in prostate weight and have been found in rapidly involuting prostate tumour transplants of castrated rats (*163*) which are undergoing apoptosis and supports apoptosis as a mechanism of prostate weight reduction. Other scenarios in which such cells are found include inflammation, infection and wound healing. These events are highly unlikely to occur in the prostates of the treated mice in this study which show no other evidence of such occurrences. However, the rate of apoptosis in the prostates of the mice in this study is below a level detectable by staining *in situ*, and thus the possibility of an alteration in some aspects of prostate development throughout mouse development remains a strong possibility.

The reduction in prostate weight with higher doses of Aroclor 1254 does not imply a clear impact on prostate function in this study. An analysis of prostatic fluid composition and ejaculated sperm morphology and motility may indicate changes in prostate function, although these tests were not included in this work. However, the

possibility still remains that Aroclor 1254 has the ability to alter the import and export of steroid hormones in prostate cells. Changes in the levels of steroid hormones such as testosterone and DHT would alter prostate cell signalling and impact organ growth. The predominance of dilated acini in the ventral prostates in a dose related manner is a relevant finding, but does not indicate functional effects. Assessment of changes in prostatic fluid proteins and proteinases were beyond the scope of this project, but could provide valuable information regarding prostate functional alterations due to PCB exposure.

Although the prostates of the 20 and 40 mg/kg/day dosed mice maintain the structures necessary for prostate function, these organs are lower in weight and show the presence of abnormal cell types. These findings may hold valuable implications for wildlife and human reproductive health and species fertility.

4.5 Future Directions

Further studies on PCB metabolism in the cell lines used would provide information regarding the action of the PCBs tested in this study and give more specifics about chemicals which have the capacity to interact with the AR. A greater understanding of the effects of CYP changes on androgen levels and the impact seen *in vitro* and *in vivo* may clarify some of the mechanisms of PCB action.

A congener-specific analysis of the mixtures tested would provide information regarding AR ligand binding characteristics and capabilities of individual components of these complex mixtures. Future *in vitro* studies examining the action of a broader spectrum of congeners in combination with ligand displacement assays would narrow the focus of the active congeners *in vitro*, and may help uncover the mechanisms of PCB alteration of hormone action in cellular systems. Incorporating experimental anaylses of

mechanisms involved in the import and export of steroid hormones and PCB congeners into the sensitive *in vitro* and *in vivo* assays that have already been established may help explain the changes in cellular steroid hormone levels in culture. These studies may also offer further information regarding pathways that lead to changes in hormone regulated organs such as the prostate.

The evidence presented here demonstrates the ability of PCBs to influence androgen regulated processes *in vitro* at the molecular level in cell culture systems as well as *in vivo* in a mammalian androgen regulated transgene in the reproductive organ system. These results warrant further investigation of how these ubiquitous organochlorines impact on other androgen influenced systems in the body. For example, in addition to the prostate, the seminal vesicles are androgen regulated and therefore likely to be sensitive to compounds that alter androgen action. During development, androgens affect Wolffian duct development during embryogenesis and stimulate and maintain spermatogenesis. The influences of androgens are also required for the functional differentiation of the hypothalamus and erythropoeisis stimulation. Finally, androgens exert anabolic growth effects on skeletal muscle development and on bones by causing the epiphyses to ossify in long bones, which inhibits linear growth. An understanding of the impact of PCB mixtures and individual congeners on androgen regulated aspects of human and animal development and function will direct us towards improving and protecting our health and reproductive fitness.

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