Effect of environmental pollutants, polychlorinated biphenyls (PCBs), on the reproductive function of adult male rhesus monkey (*Macaca mulatta*)

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By

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Certificate

This thesis by Mr. Uzair Ahmad Syed is accepted in its present form by the Department of Biological Sciences as satisfying the thesis requirements for the degree of Doctor of Philosophy in Biology (Reproductive Toxicology).

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Contents

Abbreviations

A	Aroclor
ADP	Adenosine diphospate
AHH	Aryl hydrocarbon hydroxylase
Ah	Aryl hydrocarbon
Ap	A-pale
Ad	A-dark
bw	Body weight
°C	Degree centigrade
C	Crater
CB	Chlorobiphenyl
cf	Correlation coefficient
Chol	Cholesterol
CK	Creatinine kinase
CYP	Cytochrome
DHT	Dihydrotestosterone
DNA	Deoxyribose nucleic acid
E	Estradiol-17β
ECOD	Ethoxycoumarin-o-deethylase
ER	Estrogen receptor
EROD	Ethoxyresorufin o-deethylase
ES	Ectoplasmic specialization
f	Fento
Fig.	Figure
FT4	Free thyroxin
g	Gram
G1	Growth period 1
GABA	Gamma amino butyric acid
GDA	Glutaraldehyde
GnRH	Gonadotropin releasing hormone
HDL	High density lipoproteins
HMFO	Hepatic mixed function oxidase
HPTE	2,2-bis (*p*-hydroxyphenyl)-1,1,1-trichloroethane
5-HT	5-hydroxytryptamine
i,p	Intraperitoneally
i,t	Intra testicular
IVF	*In vitro* fertilization
kg	Kilo gram
L	length
LH	Luteinizing hormone
LDL	Low density lipoprotein
M	Molar
m	Meter
n	Number
MAO	Mono amine oxidase

ml	Milli liter
NaCl	Sodium chloride
NADPH	Nictotineamide Diphosphate
ng	Nano gram
nm	Nano meter
NIOSH	National Institute of Occupational Security and Health
OH-CB	Hydroxy chlorobiphenyls
OsO_4	Osmium tetaroxide
P	Probability
PASTIC	Pakistan Scientific and Technological Center
PCB	Polychlorinated biphenyl
PCDF	Polychlorinated dibenzofuran
pg	Pico gram
POAH	Preoptic-anterior hypothalamic area
POP	Persistent organic pollutant
ppb	Parts per billion
Ppm	Parts per million
PRAP	Poly ADP-ribose polymerase
PROD	Pentoxyresorufin O-dealkylase
QAU	Quaid-i-Azam University
RER	Rough endoplasmic reticulum
RIA	Radioimmunoassay
SER	Smooth endoplasmic reticulum
SEM	Standard Error Mean
T	Testosterone
T3	Triiodothyronin
T4	Thyroxin
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TCB	Tetrachlorobiphenyl
TCDF	2,3,7,8-tetrachlorodibenzo-p-furan
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TPH	Tryptophan hydroxylase
TT4	Total thyroxin
UGC	University Grants Commission
US	United States
VLDL	Very low density lipoproteins
WHO	World Health Organization

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Uzair Ahmad Syed

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Abstract

Three groups (n=4 each) of adult male rhesus monkeys (*Macaca mulatta*) were ven oral treatment of Aroclor 1242 or Aroclor 1254 at a dose of 200 µg/kg v/day/animal or vehicle (corn oil and glycerol) for six months to examine the effect of CBs on plasma testosterone, testicular and accessory gland morphology. During the eatment period, observations were made on body mass and testicular size. Blood mples (3 ml X 2) were collected on weekly basis for determination of testosterone vels. At the end of the treatment period, the animals were sacrificed humanely. Testis id accessory glands (epididymides, seminal vesicles and prostrates) were removed, ed and processed for light and electron microscopy. Serum testosterone ncentration was determined by radioimmunoassay.

PCB treatment caused pathological symptoms (loss of appetite, diarrhea, thargy, fever and vomiting), clinical signs of toxicity (swelling of eyelids, edema under e eyes, hair loss and appearance of lesions in nail beds) and neurobehavioral anges including depression, aggression and lethargy. Mean body weights of Aroclor 254-treated animals decreased significantly while the Aroclor 1242-treatment only ghtly reduced body weights of the treated animals. Testicular diameter of both the CB-treated groups also decreased significantly during the treatment period. The anges in body weight and testicular diameter were highly correlated in Aroclor 1254-eated group but not in Aroclor 1242-treated animals. While Aroclor 1254-treatment did t affect the mean plasma testosterone levels, there was a general decline in the vels of hormone in Aroclor 1242-treated monkeys.

PCB-treatment resulted in disruption of normal epithelial organization due to ich spermatogenetic activity in the seminiferous tubules was adversely affected. The ckness of the tunica propria increased in both the PCB-treated groups and it veloped wavy margins accompanied by separation of the basement membrane from e necrotic peritubular Myeoid cell layer. The number of spermatogonia was reduced id differentiation between sub-types was not possible in the Aroclor 1254-treated

oup. The spermatogonia of Aroclor 1254-treated animals were abnormally large in ze showing much variability in shape and disorganization of cytoplasm. They had und or oval completely euchromatic and hypertrophied nuclei. On the other hand, the permatogonia of Aroclor 1242-treated testes did not show such abnormalities in size nd shape, yet exhibited slight hypertrophy and vesiculation. The Sertoli cells of Aroclor 254-treated testes appeared highly shrunken with clustering at some places. Nuclear foldings were reduced to a great extent. In Aroclor 1242-treated testes, the toplasmic extensions of Sertoli cells in many sections occupied luminal areas besides lot of fat droplets gathered peripherally. The Leydig cells of the Aroclor 1254- and ehicle-treated monkeys were more or less similar. Leydig cells of Aroclor 1242-treated stes were highly damaged and contained pyknotic nuclei. Cytoplasm was scanty. At me places, the Leydig cells also contained fat droplets and vacuolation.

The ultrastructural observations of testes revealed that the basement membrane both the PCB-treated groups was far apart from the Myeoid cells and the gap was ccupied by disassembled collagenous fibers. In Aroclor 1254-treated testes, permatogonia lacked most of the cytoplasmic components and developed empty paces. Delamination of the cell membrane, distortion of mitochondrial cristae, ustering of mitochondria, decreased endoplasmic reticulum, shrinkage of the toplasm and pyknosis of nuclei were the main features of spermatogonia of Aroclor 242-treated testes. In Aroclor 1254-treated testes, the spermatocytes were very rare nd highly necrotic. However, in Aroclor 1242-treated testes, though the spermatocytes ad condensed chromatin, yet the cytoplasm at most of places was shrunken. The ocess of spermeogenesis which was not evident in the sections of Aroclor 1254-eated testes, was also adversely affected in Aroclor 1242-treated testes exhibiting amage in the form of shrinkage of the cytoplasm and thickening of cell membrane of e round and elongated spermatids, absence of stages of acrosomal formation and istence of some abnormal spermatozoa. In the Aroclor 1254-treated testes, the size the Sertoli cells was much reduced, cytoplasm was disorganized and lacked ganelles except mitochondria, ectoplasmic specializations were damaged and nuclear foldings were reduced. Though the cytoplasm of the Sertoli cells of Aroclor 1242-

rated testes contained mitochondria, endoplasmic reticulum, Golgi apparatus and fat oplets, empty spaces of variable sizes and reduction of nuclear infoldings were mmon observations in these cells. In the Leydig cells of the Aroclor 1242-treated imals, the cytoplasm developed zones of electron dense and electron opaque gions, empty patches, thread-like extensions of the cell membrane, distortion and knosis of nuclei and variations in the quantity of heterochromatin.

All accessory glands were also affected by the PCB treatment. The proportion of nnective tissue increased considerably in all the PCB-treated accessory glands. The ithelium in the Aroclor 1254-treated epididymides was increased in thickness and ratification. The cells were necrotic and lacked stereocilia. It was not possible to scriminate between cell types owing to the effect of the pollutant. Some of the cells ere abnormally large. In the epididymides of the Aroclor 1242-treated animals, the minal spaces lacked spermatozoa. The epithelium contained irregular-shaped necrotic lls that possessed stereocilia. In the prostate glands of the Aroclor 1254-treated imals, the lumina of the gland were either collapsed or much reduced in size and ed with a single layer of necrotic cells with pyknotic nuclei that frequently showed tachment and accumulated in the luminal matrix. In the prostate glands of the Aroclor 242-treated animals, the epithelium was thickened with cells that had lost their normal boidal/columnar shape. The nuclei of these cells too were pyknotic. Often the ithelial cells were hypertrophied and thus appeared conspicuous. In the seminal sicles of both the PCB-treated groups, the mucosal fold attained abnormal ganization with an increase in the thickness of the epithelium due to increased ratification. Cellular features of PCB-treated seminal vesicles were also adversely fected.

It is concluded that exposure of male adult rhesus monkeys to Aroclor 1254 and oclor 1242 causes severe morphological alterations. Aroclor 1254 has far more totoxic influence than Aroclor 1242. Of the two PCBs tested on monkeys, only Aroclor 42 exerted estrogenic effect.

INTRODUCTION

Biological hazard assessment of environmental pollutants is an important aspect of environmental medicine as the anthropogenic chemicals used indiscriminately by man have not only threatened human and wildlife health and reproduction but may have dire consequences leading to ecological catastrophe in some parts of the world. Of these pollutants, polychlorinated biphenyls (PCBs) have contaminated almost all parts of the globe. As highly persistent contaminants of air, water and soil, PCBs bioaccumulate in wildlife and humans where they are well known to cause cancer and developmental abnormalities, disrupt reproduction and endocrine functions besides exhibiting pathological symptoms in many body parts. PCBs are known to alter reproduction in humans, wildlife and experimental animals at various levels. These chemicals adversely affect puberty, fertility, reproductive cycles, fertilization, mortality, and growth of offspring.

PCBs are mixtures of congeners whose empirical formula is $C_{12}H_{10-1}Cl_n$ (n=1-10). A PCB molecule (congener) consists of a biphenyl nucleus with chlorine at any one or all of the 10 available sites of which ortho, meta and para positions are important for chlorine substitution and determine the properties of the molecule (Fig. 1). Thus 209 different PCB congeners are theoretically possible. PCB molecules assume a planar conformation with increasing chlorine substitution, determined primarily by the number of ortho substituents. The number of ortho substituents is used to name each class of PCB congeners. The term co-planar is applied to congeners with no substitutes at the ortho positions. Other groups of PCB congeners are referred to as mono-, di-, tri- or tetra-ortho substituted congeners (Battershill, 1994).

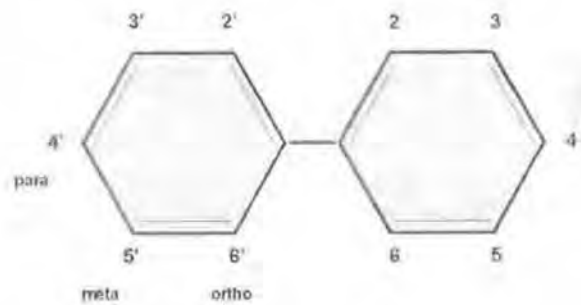


Fig. 1. Structure of PCB molecule

These compounds were widely synthesized during 1930-1970 by a number of manufacturers (Table 1). Aroclors are usually given a four-digit number in which the first two digits refer to the number of carbon atoms and the last two indicate the percentage (by weight) of chlorine (Nessel and Gallo, 1994). Aroclor 1254 is a mixture of 3,3′, 4,4′-tetra-, 3,3′, 4,4′, 5 penta- and 3,3′, 4,4′, 5,5′-hexachlorobiphenyl congeners (Mes *et al.*, 1995). Major constituents of Aroclor 1242 are 2,2′ 4,4′-tetra- and 2,2′ 4,4′ 5,5′-hexa-chlorinated biphenyls. Both the Aroclors contain small quantities (0.15-5.6 mng/kg) of polychlorinated dibenzofuran (PCDF) (Battershill, 1994).

Table 1. PCB Trade names and manufacturers

Trade name	Trade name Owner
Aroclor	Monsanto Company St. Louis, MO, USA
Chlorextol	Allis-Chalmers Milwaukee, WI, USA
Clophen	Farbenfabricken Bayer GmbH, Germany
Dykanol	Federal Pacific Electric Co. Newark, NJ, USA
Fenclor	Caffaro S.P.A., Italy
Inerteen	Westinghouse Electric Corp. Pittsburgh, PA , USA
Kanechlor	Kanegafuchi Chemical Industry Co., Ltd., Japan
Noflamol	Wagner Electric Corporation, Newark, NJ, USA
Phenoclor	Prodelec, France
Pyralene	Prodelec, France
Pyranol	General Electric Co., Schenectady, NY, USA
Santotherm	Mitsubishi-Monsanto, Japan

(Source: NIOSH)

PCB mixtures are very resistant to degradation by oxidation, acids, bases, and other chemical agents. These chemicals are good electrical insulators and are thermally stable. These are soluble in most of the common organic solvents and lipids, but are only slightly soluble in water, glycerol and glycols. Although most individual PCBs are solid at room temperature, their mixtures vary in viscosity from oils to sticky resins (Durfee *et al.*, 1976). PCBs, have remained in wide use in industry as fluids for heat transfer and hydraulic systems, gas turbines, vacuum pumps, fire retardants, plasticizers in adhesives, textiles, surface coatings, sealants, printing, carbonless copy paper, air conditioners, and fluorescent lamp fixtures. PCBs have also been used in capacitors and electric transformers. More than 95% of all power capacitors contain these chemicals. Approximately each transformer contains between 40 and 500 gallons of PCBs (Nessel and Gallo, 1994).

Enormous quantities of PCBs have been released into the environment as a result of improper disposal practices and accidental releases. Their lowest residue levels have been reported from Antarctica and the highest from the northern hemisphere (Kamrin and Ringer, 1994). PCB concentrations in the environment are reported to be higher (1-10 ng/m^3) in urban and heavily industrialized areas than (0.1-0.5 ng/m^3) in rural sites (Kimbrough, 1987; Atlas *et al.*, 1986). PCB concentrations in soils range from 10 ppm to over 100 ppm (Tatsukawa, 1976) and are detected in soil samples from both metropolitan areas and agricultural lands (Carey and Gowen, 1976). These compounds are strongly absorbed in surface soil constituents (Chou and Griffin, 1986) and upon contact penetrate the skin (Wester *et al.*, 1993). PCB concentrations in the environment are influenced by such factors as local sources, emission strengths, and meteorological conditions. Fires in buildings and installations are one of the major sources of PCB dispersion in the environment. PCB concentrations were detected in wild animals not only after a fire in St. Basile le Grand, Quebec, but the concentration of PCBs increased in these animals even 1-10 months later (Phaneuf *et al.*, 1995).

PCBs are found in measurable concentrations in many food items (Himberg, 1993). In some parts of the world export meat samples from chicken or pork, are found to contain more than 50 ng/g PCBs. Part of this contamination stems from imported animal feed ingredients (fish flour and grains). However, after comparing PCB concentrations in fish flour and grains with those found in meat, it has been found that the high concentrations stem from recycled fat (Schepens *et al.*, 2001). Youngsters could have a higher intake, due to the presence of PCBs in cow's milk, butter and cheese, and food items with a mixture of vegetable and animal fats and oil, as added by the food industry (Theelen *et al.*, 1993).

Elevated concentrations of these compounds are detected in workers involved in capacitor manufacture, hazardous waste disposal work (Luotamo *et al.*, 1993), the transformer industry and municipal incineration (Schecter *et al.*, 1994). Because of their lipophilic nature, PCBs are often found in human breast milk (Truelove *et al.*, 1982) where their concentration has been reported up to 5.5 ng/g (Hashimoto *et al.*,

1995). It has become evident from a study in which samples of mother's milk collected during 1990/91 were compared with those of 1984/85 that the high-chlorinated congeners were equally persistent and the low-chlorinated congeners tend to even increase (Georgii *et al.*, 1993). Concentrations of PCBs have been found to be higher in the milk samples of Canadian women who consumed greater than 100 g of fish weekly (Newsome *et al.*, 1992). *In utero* and breast milk PCB exposure studies revealed that on lipid basis, the concentration of PCB in placenta was 2.8 times higher than in breast milk which shows that PCBs may be capable of crossing the placenta to a greater extent (DeKoning and Karmaus, 2000).

PCBs accumulate in human and animal tissues as these are poorly metabolized (Biros *et al.*, 1970; Yobs, 1972: Finklea *et al.*, 1972; Kimbrough, 1974). The accumulation particularly in tissues and organs rich in lipids, appears to be higher in the case of *penta* and more highly chlorinated biphenyls (Hutzinger *et al.*, 1974). However, some congeners show a lower bioaccumulation potential because they can be systematically metabolized during their passage through the food chain. Hydroxylated metabolites of congeners are metabolized through conjugation to glutathione to finally methylsulfones via the mercapturic acid pathway, involving the enterohepatic cycle (Boon *et al.*, 1997).

PCBs exert their toxic effects in a number of ways of which the most widely accepted mechanisms are as follows:

Ah receptor mediated toxicity:

Members of the PCB family can bind to cytosolic aryl hydrocarbon (Ah) receptors, which undergo transformation and translocation into the nucleus occupying a nuclear binding site and alter gene expression (Safe, 1994). Their binding affinity depends on the degree of chlorination of the molecule and the position of the chlorine atoms (Tryphonas, 1994). The Ah receptor functions as a transcriptional enhancer, interacting with a number of other regulatory proteins such as Aryl hydrocarbon hydroxylase, heat shock proteins, DNA binding proteins, kinases, translocases etc. Interactions with specific base sequences in the DNA appear to be modulated by the presence of other growth factors, hormones, and

their receptors as well as other regulatory proteins (Battershill, 1994). Structure-function relationships for PCB congeners have identified two major structural classes. These are: Coplanar PCBs that mediate their effects through Ah receptors. In contrast, congeners possessing chlorine substitution at the ortho positions of the biphenyl rings are non-coplanar and do not bind with high affinity to the Ah receptor (Agrawal *et al.*, 1981; Eriksson *et al.*, 1991; Ku *et al.*, 1994).

A number of the effects observed for the commercial PCBs are similar to those reported for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. This has led to the establishment of toxicity equivalency factors (TEFs) that relate the potency of individual polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and polychlorinated biphenyl (PCB) congeners to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Mes, 1993; Zabel *et al.*, 1995). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) commonly referred to as **"dioxin"** exerts its effects through interaction with the aryl hydrocarbon (Ah) receptor. It brings about a chain of events leading to various responses including enzyme induction, immunotoxicity, reproductive and endocrine effects, developmental toxicity, chloracne and tumor promotion. While Ah receptor variants exist, all vertebrates examined have demonstrated such a protein with similar numbers of receptors and binding affinity for TCDD (Fischer *et al.*, 1998). The toxic equivalency (TEQ) of a mixture is determined by summing the products of each component times its equivalency to TCDD (Hansen, 1998).

Estrogenecity:

The natural estrogen, estradiol-17β (E), is involved in gonadotropin regulation of Leydig cell steroidogenesis, fluid absorption in the male reproductive tract and maintenance of male bone density. Estrogen receptors are expressed in fetal, neonatal, and adult tissues, including the hypothalamus, pituitary, testis and the ex-current duct system suggesting that estrogen may support multiple activities in the male reproduction. Leydig cells in the testis produce the primary male hormone, testosterone, almost exclusively. Leydig cell differentiation is sensitive to inhibition by estrogen (Akingbemi *et al.*, 2000). A number of environmental contaminants

including PCBs termed as xenoestrogens have capacity to disturb the working of natural estrogens in a number of different ways. They can;

(1) bind to specific estrogen receptor sites, preventing the hormone from binding with the site thus blocking or inhibiting the proper hormone response;
(2) bind to other receptors and create a novel reaction or interfere indirectly with normal hormonal action, and
(3) alter production and breakdown of hormone receptors and natural hormones, which changes hormonal blood concentrations and endocrine responses

Thus the persistence of xenoestrogens and their metabolites in the environment pose a risk to both human and wildlife populations by altering reproduction and promoting neoplasia. Members of the PCB family are also reported to exert estrogenic effects. In extreme cases, these compounds are found to alter sexual differentiation that lead to gonadal sex reversal in a reptilian species that exhibits temperature-dependent sex determination (Bergeron *et al.*, 1994).

Induction of enzymes:

One possible mechanism through which PCBs intoxicate is by inducing certain enzymes, thus altering ultimate responses through their influence on enzyme levels and enzyme kinetics (Soontornchat *et al.*, 1994). One of the most readily induced enzymes by PCBs is the cytochrome P450 family, members of which are involved in the metabolism of steroid hormones (Yawetz *et al.*, 1993; Chen *et al.*, 1994) such as they increase in androstenedione levels and decrease testosterone levels (Machala *et al.*, 1998; Akingbemi *et al.*, 2000; Andric *et al.*, 2000). Analysis of the kinetics and congener selectivities for PCB metabolism in occupationally exposed workers shows that the PCB concentrations required for the induction of P_{4502B} cytochromes must be >20 mg/kg and for inducing $P450_{1A}$ and their associated toxic effects, it must be >600 mg/kg (Brown *et al.*, 1994). The observed induction of cytochrome P450 species in PCB-exposed populations of various animal species suggests that these enzymes can be used as bioindicators for PCB pollution monitoring (Brown *et al.*, 1994).

Besides Cytochrome the P450 family, PCBs can also induce other enzyme systems including malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the liver (Hitomi, 1993), steroid-metabolizing enzymes such as ethoxyresorufin o-deethylase (EROD), ethoxycoumarin o-deethylase (ECOD) (Olson *et al.*, 1999) and hepatic mixed function oxidase (HMFO) (Bhattia *et al.*, 1994).

Endocrine disruption:

Synthetic chemicals and natural plant compounds originating outside the body that have a hormone-like activity in the body are referred to a variety of terms: endocrine-disruptors. Reproductive system depends on the normal functioning of endocrine/ neuroendocrine systems. PCBs also disrupt endocrine functions at various levels. These chemicals induce changes in brain neurochemistry (Shain *et al.*, 1991; Seegal and Shain, 1992; Seegal and Schantz, 1994), catecholamine release and contents of primary cultures of bovine chromaffin cells (Messeri *et al.*, 1993) and ontogeny of rat monoamine oxidase and acetyl cholinesterase (Vincent *et al.*, 1992). Ortho-substituted PCBs inhibit dopamine uptake and transmitter transport into synaptosomes from rat brain (Mariussen *et al.*, 2001). The uptake of dopamine, glutamate, GABA and serotonin is inhibited by the PCB mixtures, Aroclor 1242 and 1254 (Mariussen and Fonnum, 2001).

PCBs alter thyroid function by decreasing hormone secretion and changing histological features of thymocytes in various animals (Chang *et al.*, 1980; Byme *et al.*, 1987; Meserve *et al.*, 1992; Morse *et al.*, 1993; Visser *et al.*, 1993; Goldey *et al.*, 1995; Hansen *et al.*, 1995; Morse *et al.*, 1996b). PCBs also produce remarkable degeneration, necrosis and disarray in the adrenal cortex and medulla (Rao and Banerji, 1993). One possible way by which PCBs may interfere with endocrine function is their ability to mimic natural hormones such as those of thyroid and endocrine steroids (McKinney and Waller, 1994).

Although PCB exposure has been associated with reduced reproduction in wildlife, definitive cause-and-effect data are lacking. Whether human exposure to the PCBs is sufficiently great to produce serious effects such as reproductive

insufficiency has not been fully determined. Following the initial experimental studies with non-human primates, Allen *et al.*, (1973 & 1974) suggested that humans and non-human primates responded similarly to PCB exposure. PCB toxicity studies have been carried out in various animals groups and their toxic effects are reported in almost all vertebrate groups including primates. In spite of the data gathered as a result of PCB toxicity studies a part of which have been described here, the effects of PCBs on male reproduction in mammals are less studied. Specifically, the structural alterations caused by the PCBs on mammalian reproductive organs have not been undertaken. The current study was therefore, undertaken to assess the effects of PCB mixtures (Aroclor 1254 and Aroclor 1242) on the male reproductive function of adult rhesus monkeys (*Macaca mulata*) so as to assess the effects on morphological and physiological aspects.

REVIEW OF LITERATURE

Study of PCB toxicity started in early 1970's with special focus on the reproductive system of fish, birds and mammals.

PCBs have adverse effects on hatchability of eggs and survival of fry of such fish species as fathead minnow (*Pimephales promelas*; Nebeker *et al.*, 1974), coho salmon (*Onchorhynchus kisutch;* Halter and Johnson, 1974), brook trout (*Salvelinus fontinalis;* Snarski and Puglisi, 1976; Mauck *et al.*, 1978), sheephead minnow (*Cyprinidon variegates;* Schimmel *et al.*, 1974), Atlantic cod (*Gadus morrhua;* Freeman *et al.*, 1982) and mummichog (*Fundulus heteroclitus;* Weis and Weis, 1982). Besides affecting fecundity, PCBs have also been found to exert adverse influences on testicular function by causing derangement of lobules, hyperplasia of lobule walls and necrosis of spermatogenetic components (Sangalang *et al.*, 1981). Exposure of Atlantic croaker (*Micropogonias undulatus*) to Aroclor 1254 (1 μg/kg bw/day) for 30 days during the early-recrudescence phase of the gonadal cycle results in the impairment of LH secretion, gonadal growth and GnRH secretion in the preoptic-anterior hypothalamic area (POAH). During testicular maturation this treatment decreases the number of pituitary GnRH receptors, perhaps due to an impairment of GnRH release resulting from either due to a direct action of PCB on GnRH neurons or indirectly via interference with other neurotransmitter pathways that modulate GnRH function (Khan *et al.*, 2001).

In birds, PCBs influence reproduction through adverse effects on egg production (Platonow and Reinhart, 1973; Call and Harrell, 1974; Stendall, 1976), thickness of egg shell (Cooke, 1973), embryos (Platonow and Reinhart, 1973; Cecil *et al.*, 1974; Brunstrom and Darnerud, 1983), survival (Koeman *et al.*, 1969; Platonow and Funneli, 1971; Dahlgren *et al.*, 1972; Harris and Rose, 1972; Hurst *et al.*, 1973; Hill *et al.*, 1975; Holleman *et al.*, 1976), and behavior (Fisher *et al.*, 2001; Fernie *et al.*, 2001).

PCBs are concentrated in food chains in the marine environment and reach highest concentrations in marine mammals as top predators (Tanabe and Tatsukawa, 1991). Male marine mammals continue to accumulate PCBs throughout their lives, while the females generally transfer a large part of their burden to their progeny during gestation and lactation. The lipophilic nature of PCBs facilitates such transfer from mother to the offspring (Tanabe, 1994; Addison and Brodie, 1997). In the female common seal (*Phoca vitulina*) fed fish contaminated with PCBs, conception and implantation of the blastocyst is disturbed. Also plasma thyroxine, triiodothyronin and retinol levels decline (Reijnders, 1986; Brouwer *et al.*, 1989). The litter size is significantly reduced in the mink (*Mustela vison*) fed with PCB contaminated fish (Aulerich and Ringer, 1977) or following oral administration of PCB (Jensen *et al.*, 1977; Wren *et al.*, 1987b).

Rodents have been widely used in PCB toxicity studies. In the male house mouse (*Mus musculus*), administration of Aroclor 1254 at 200 ppm in the diet for 15 days reduces testicular sperm content without affecting testis weight (Sanders *et al.*, 1977) and administration of 1000 ppm for 2 weeks reduces androgen-dependent seminal vesicle weight (Sanders *et al.*, 1974; Coffey, 1988). Similar effects occur in the white-footed mouse (*Peromyscus leucopus*) after they are fed Aroclor 1254 at a dose of 400 ppm for 2 weeks (Sanders and Kirkpatrick, 1975). Oral administration of Aroclor 1242 at a concentration of 25 mg/kg bw to female mice every 2-days from 2-weeks before mating, during mating, and through gestation until postnatal day 21 increased sperm count by 36%, and epididymal sperm velocity and linearity at 16 weeks of age. However, at 16 weeks of age, sperm fertilizing ability *in vitro* significantly decreases in all PCB-exposed groups at 16 and 45 weeks of age (Fielden *et al.*, 2001). Aroclor 1254-treatment to female rats at a dose of 32 or 64 mg/kg bw causes decrease in testicular, prostate and seminal vesicle weight in as old as 165 days offspring (Sager, 1983). Continuous exposure of lactating female rats to PCBs increases testis weight, sperm production and Sertoli cell proliferation in the adult male offspring (Cooke *et al.*, 1996; Kim, 2001). However, this is an indirect effect. Perhaps, PCBs cause hypothyroidism and neonatal hypothyroidism if caused during a specific period of development produces this effect (Cooke *et al.*, 1996).

Aroclor 1254 administration to female mice prior to mating till day 8-10 of gestation causes failure of blastocyst implantation (Orberg and Kihlstrom, 1973). Trans-lactational exposure of rats to Aroclor 1254 not only delays puberty in the female offspring but also results in delayed decrease in uterine response, impairment of fertility and irregular cycle patterns (Sager and Girard, 1994). Aroclor 1254-treatment to female rats at a dose of 32 or 64 mg/kg bw causes decrease in testicular, prostate and seminal vesicle weight in as old as 165 days offspring. The female offspring experience greater misconception and resorption rates (Sager, 1983). Various PCB mixtures have been reported to alter estrous cycles in the mouse (Orberg and Kihistrom, 1973) and rats (Bitman and Cecil, 1970; Gellert, 1978). One month exposure of Aroclor 1254 at a dose of 10 mg/kg bw to rats causes prolongation of estrous cycles, decrease in sexual receptivity, delay in timing of copulation and vaginal bleeding during copulation (Brenzer *et al.*, 1984). Rats produce small-sized dams when exposed to Aroclor 1254 at a dose of 20 mg/kg bw and the number of litters decreases to a great extent when the dose is increased to 100 mg/kg body weight. Even the surviving animals fail to produce live offspring (Linder *et al.*, 1974). Large doses of Aroclor 1242, given intraperitoneally to immature female Sprague Dawley rats, cause an increase in absolute uterine weight and decrease pentoxyresorufin O-dealkylase (PROD) activity (Soontornchat *et al.*, 1994). Administration of a coplanar PCB congener, 3,3',4,4',5,5'-hexachlorobiphenyl, on day 1 of gestation causes severe effects on reproductive capacity and sexual behavior in both males and females, even when the male and the female offspring are paired with untreated partners. (Smitsvanprooije *et al.*, 1993). Exposure of lactating rats to PCB congener, 3,3',4,4',5-pentachlorobiphenyl, *in utero* causes fetotoxic effects, delayed physical maturation, and induction of liver xenobiotic metabolizing enzymes (Bernhoft *et al.*, 1994).

A number of studies have been conducted on monkeys that have gathered very useful data regarding the toxicity of PCBs in the non-human primates. In monkeys, the half-life of the important PCB congeners ranges from 0.3-7.6 years. After exposure, these toxicants are eliminated gradually from the body. However, the mono-ortho substituted PCBs are extremely persistent (Mes *et al.*, 1995). Analysis of

PCB (Aroclor 1254) on prenecropsy blood samples and in postmortem adipose tissue, liver, kidney, and brain of rhesus monkeys has revealed that the pollutant's levels in all of these tissues increase in a dose-dependent manner (Tryphonas, 1986)

In the juvenile rhesus monkeys, administration of PCBs results in decrease in body weight gain, reduced food consumption, anemia, alopecia, subcutaneous edema of the face, swelling of eyelids, erythema, and acniform lesions (Abrahamson and Allen, 1973). The clinical symptoms of PCB exposure in adult monkeys include facial edema, alopecia, periorbital edema, congestion of the eyes, swelling of the eyelids, loss in body weight or decreased weight gain, loss of hair, acne form lesions, erythema, white secretions from the eyes, keratinization of the affected hair follicles, lesions in nail beds, separation of nails, emaciation and lacrimation (Becker *et al.*, 1979; Ohinishi and Kohno, 1979; Allen *et al.*, 1974a; Barasotti *et al.*, 1976; Hori *et al.*, 1982; Arnold *et al.*, 1984; Tryphonas *et al.*, 1984).

In monkeys, PCB exposure also exerts toxic effects on the hematological and lymphoreticular systems including immunosuppression, greater tendency for incidence of anemia, hypoprotenemia, bone marrow depletion and cytoplasmic vacuolation in erythroid precursor cells (Hori *et al.*, 1982; Tryphonas *et al.*, 1986a). PCBs are known to cause increase in plasma triglycerides and decrease in plasma total cholesterol, high-density lipoprotein cholesterol (HDL-chol), very low density cholesterol (VLDL-chol), low-density lipoprotein cholesterol (LDL-chol), and total carnitine (Bell *et al.*, 1994).

PCBs also cause histopathological changes in various organs of monkeys. Exposure to Aroclor 1242 at a dose of 3-10 mg/kg for 2-3 months causes arrest in differentiation of generative cells of the isthmus and neck region into parietal and zymogenic cells. In the rarely differentiated parietal and zymogenic cells, abnormalities are observed in the endoplasmic reticulum, mitochondria, luminal membranes and autophagic vesicles (Becker *et al.*, 1979). Lesions and metaplasia in sebaceous glands, nail-beds, gastric mucosa and ameloblast surrounding unerupted teeth develop in rhesus monkeys 13 months after exposure of diet

containing 400 mg/kg Aroclor 1242 for forty days (McNulty, 1985). Female rhesus monkeys fed a diet containing 25 mg/kg Aroclor 1248 for 2 months develop hypertrophy and hyperplasia in the muscularis mucosa and ulceration in the gastric mucosa (Allen *et al.*, 1974). Hyperplasia and keratinization of hair follicles, necrosis and enlargement of hepatocytes are observed in the female rhesus monkeys administered Aroclor 1248 at a dietary level of 2.5-5 mg/kg (Barasoti *et al.*, 1980). Dilation of tarsal gland ducts, atrophy in the splenic and lymphonodal germinal centers, gingival erosion and ulceration, mucinous hypertrophic gastropathy with cystic dilation of gastric glands, hepatocellular enlargement and necrosis, hypertrophy and hyperplasia of biliary duct epithelium, hypertrophy of gall bladder epithelium and increase in the number of lysosomes in the thyroid follicular epithelial cells have been observed by Tryphonas *et al.*, (1986a) when they exposed rhesus monkeys to Aroclor 1254 at a dose of 200 μg/kg bw for 27-28 months. In another study on cynomolgus monkeys, Hori *et al.* (1982) have noted cytoplasmic vacuolation and dilation of the convoluted tubules along with cytoplasmic cysts in the kidneys, in addition to the changes described above, when they administered monkeys with Kanechlor 400 at a dose of 4 mg/kg bw for 20 weeks. Aroclor 1254-treatment of female rhesus monkeys at doses (0, 5, 20, 40, 80 μg/Kg bw) over an extended period of six years cause histopathological lesions in adrenal gland (adrenalitis, calcification), liver (necrosis, hepatitis, cirrhosis), mammary glands (cyctic disease, dialated ducts, lobular hyperplasia, atrophy), pancreas (duct epithelial hyperplasia, enlargement of islets, decrease in the number of islets), thyroid (thyroiditis, colloid cyst, C-cell hyperplasia, enlargement of follicles, cyst), uterus (adenomyosis) and vagina (adenosis). Besides, this treatment also causes neoplasia in ovaries, fallopian ducts, uterus, kidney, breast, adrenal and pancreas (Arnold *et al.*, 1997).

Female rhesus monkeys exposed to a diet containing Aroclor 1248 at doses, 2.5 or 5.0 mg/kg bw for 6 months experienced increased menstrual bleeding and duration of cycle, flattening and prolongation of progesterone peak even after 1 month from start of the treatment, gave birth to offspring with decreased weights and stature and the offspring also exhibited clinical signs of toxicity and histopathology of various organs (Allen *et al.*, 1980). Treatment of the animals with 4 mg PCB

(Clophen A30)/kg/bw/day after ovulation significantly decreases ovulation rate in the next cycle in rhesus monkeys indicating inadequate estrogen secretion as judged from histopathological abnormalities in the primary follicles.

Aroclor 1254-treatment of female rhesus monkeys adversely affects their impregnation by the untreated males, significantly decreases dose-dependent conception rate and increases fetal mortality irrespective of age of the animals (Barasoti and Van Miller, 1984; Arnold *et al.*, 1995). Besides, it also causes developmental problems, including abortions and immunologically impaired dam births in impregnated monkeys (Barasoti *et al.*, 1976; Truelove *et al.*, 1982). Although fetal mortality is also associated with endometeriosis in monkeys, it has been difficult to associate with Aroclor 1254-treatment (Arnold *et al.*, 1996). Even low dietary levels of PCBs have been reported to disturb menstrual cycle and cause excessive and prolonged menstrual bleeding. In addition, animals that have conceived show a higher incidence of resorption and early abortion. When females are given PCBs in their diets they invariably have much higher levels of urinary ketosteroids than are detectable in the urine of the control monkeys (Barsotti & Allen, 1975) and give birth to reduce sized progeny (Kuratsune *et al.*, 1972). Such infants possess shorter bones, smaller head circumference and reduced crown-to-rump lengths (Allen *et al.*, 1974).

Human exposure to various PCB-contaminated environments especially in workers has been associated with skin disorders, cancers, immune dysfunction, behavioral changes and reproductive and developmental abnormalities, acne-like skin eruptions (chloracne), pigmentation of the skin and nails, excessive eye discharge, swelling of eyelids, and distinctive hair follicles along with systemic effects such as digestive disturbances, edema of the face and hands, burning of the eyes, impotence and hematuria (Hutzinger *et al.*, 1971; Kimbrough, 1974).

Human populations became victims of PCB toxicity accidentally on a number of occasions. In an outbreak of poisoning in Japan in 1968, over 1,000 people ingested PCB-contaminated rice bran oil for a period of several months. The incident occurred when heat transfer pipes immersed in the oil (estimated 1,500 to 2,000

ppm) developed pin-sized holes. The clinical aspects of the poisoning included chloracne, brown pigmentation of the skin and nails, distinctive hair follicles, increased eye discharge, swelling of eyelids, transient visual disturbance, and systemic gastrointestinal symptoms with jaundice (Kuratsune *et al.*, 1971). In some patients, the symptoms persisted even 3 years after PCB exposure. Stillbirths to PCB exposed women were also reported and infants born to poisoned mothers had decreased birth weights, and showed skin discoloration due to PCB placental passage (Miller, 1971). In a similar disaster, Taiwanese mothers became exposed to heat-degradation products of PCBs by ingesting contaminated rice oil in 1979 and developed Yu-Cheng ("oil-disease"). Children of these mothers were born growth retarded, with dysmorphic physical findings, and delayed cognitive development compared with the unexposed children (Guo *et al.*, 1995). Communities in Japan also developed toxicity symptoms (Yusho) by ingesting polychlorinated dibenzofuran and PCB-contaminated rice oil and exhibited dermal and ocular lesions, irregular menstrual cycles, altered immune responses and disrupted endocrine activity (Schecter *et al.*, 1994). The most tragic aspect of Yusho and Yu-Cheng diseases was that *in utero* and lactational exposure of the offspring led to poor cognitive development. Intellectual impairment was also observed in children born to women who had eaten fish contaminated with PCBs in the United States (Aoki, 2001).

PCBs have also been reported to cause breast cancer. However, significant differences have been observed in the etiological role of various PCB congeners. The PCB congener, 2,2',3,4,4',5',6-heptachlorobiphenyl, has been found to be significantly associated with breast cancer risk in women with adipose levels of >5.67 ng/g PCB (Stellman *et al.*, 2000). Chinese PCB3 at 7.8 pg/ml and 182 pg/ml concentrations shows 94% and 86% of relative cellular proliferation respectively and Chinese PCB5 at 8.3 pg/ml concentration also shows 107% relative cellular proliferation as compared to 17 beta-estradiol. Thus, both PCBs seem to be different from the corresponding Aroclor mixtures. However, Chinese PCBs do not express cell proliferation effects at higher levels of 9.1 ng/ml (Chinese PCB3) and 166 pg/ml and 8.3 ng/ml (Chinese PCB3). This may be due to cytotoxicity and/or antiestrogenic compounds in the mixtures (Du *et al.*, 2000).

MATERIALS AND METHODS

Chemicals and reagents:

The polychlorinated biphenyls used in this study were Monsanto Electrical Grade Aroclor 1254 (Lot KB05-612) and Aroclor 1242 (Lot KB05-415) (Monsanto Company, St Louis, MO, USA). The reagents for testosterone radioimmunoassay (RIA) were received from World Health Organization (WHO) under the RIA Reagent Programme. Glutaraldehyde (GDA) and Osmium tetroxide (OsO_4) were purchased from Sigma Chemical Co., St Louis, MO, USA and the Epoxy embedding resin (LX-112) was purchased from LADD Research Industries Inc., USA.

Animals and dosing:

Twelve healthy adult male rhesus monkeys (*Macaca mulatta*) were used in this study. These animals were purchased from local suppliers who captured these animals from the wild habitats of Northern Pakistan. Soon after their arrival in the Primate Facility of the Quaid-I-Azam University, the animals were given a thorough wash and were housed in individual stainless steel cages under standard colony conditions (temp; 24±2°C, 12:12 light/dark cycle).

The animals were fed on a diet containing bread, beans, carrot, apple and banana. The age of animals was determined by dentition formula. The animals were quarantined for 2 months during which they were also habituated to self-ingest gelatin capsules that were later used for oral administration of PCBs.

For 6-months treatment period, animals were divided into three groups of four each. During treatment, one of these groups was given Aroclor 1254 mixed with vehicle at a dose of 200 μg/kg/day/animal, the second received Aroclor 1242 mixed with vehicle at a dose of 200 μg/kg/day/animal and the third (control group) received only vehicle (corn oil-glycerol mixture).

ionitoring, blood sampling and euthanasia:

During the treatment period, body weight / testicular measurements and blood ampling were made on the treated and control animals anesthetized with ketamine ydrochloride every week. Body weights of the treated and control animals were ieasured by using a balance at grams precision. Testicular measurements were made sing a vernier caliper at length (L) and crater (C). Testicular diameter was then alculated with the following formula:

$$\text{Testicular diameter} = L\,(C^2)\,\pi/6$$

Weekly blood samples (3 ml X 2) were collected from each animal by enipuncture following sedation by ketamine hydrochloride. At the end of the treatment eriod animals were sacrificed humanely (euthanasia; sodium penta barbital). The estes and accessory glands (epididymides, seminal vesicles and prostates) were emoved, cleared of excessive tissues and weighed at milligram precision.

)etermination of plasma testosterone

Plasma concentrations of testosterone were determined in duplicate by using pecific radioimmunoassay (RIA) reagents supplied by WHO's Special Programme of .esearch in Human Reproduction. Testosterone assays were performed on ether xtracts of plasma without chromatographic separation. The extraction recovery for the teroid was >85%. Aliquots of 40 µl of plasma were extracted with 5 ml of Anesthetic ther, taken to dryness under air in a water bath at 60 °C and then reconstituted with ppropriate volumes of assay buffer (0.1 M phosphate buffer, 0.9% NaCl, 0.1% gelatin nd 0.1% sodium azide; pH 7.2). The samples and the standards (500 µl) were ıcubated separately with antibody (100 µl) and tritiated steroid (100 µl; [1,2,6,7 H] :stosterone) for 18 hours at 4°C. Following incubation, the tubes were placed in ice nd 200 µl of dextran-coated charcoal was added to each tube. The tubes were then ept at 4°C for 30-35 min before centrifugation at 3000 rpm for 10 min. The clear ıpernatant was decanted into scintillation vials containing 5 ml of scintillation fluid (0.5) Permablend III containing ppo 5.0 % tris-MSB in toluene; Packard International, urich. Switzerland). Radioactivity was measured in a Beckman liquid scintillation

ounter (LS 1801). The results of RIA were calculated according to the WHO mmunoassay Processing Programme. The intra and inter-assay coefficients of ariation were 4.0 and 11.0 %, respectively.

ight and Electron microscopy:

Tissues were fixed in 2% phosphate-buffered Glutaraldehyde (GDA, pH 7.2), ost-fixed in 1% Osmium tetroxide (OsO_4), dehydrated in ascending acetone series, eared in propylene oxide and embedded in epoxy resin (LX-112, Ladd Research ndustries, USA). Semithin and ultrathin sections of tissue blocks were made on LKB ltratome using glass knives. Semithin sections were stained with 1% toluidine blue and tudied under a Nikon Optiphot light microscope. The ultrathin sections were transferred copper grids and contrasted sequentially with uranyl acetate and lead citrate. bservations were made on a JEOL SX 100 Transmission Electron Microscope.

istological measurements:

Diameters of the seminiferous cords, spermatogonial size and nuclear diameter f spermatogonia were measured with a pre-calibrated ocular micrometer in 20 selected ections of each testis of the Aroclor 1254, Aroclor 1242-treated and control animals.

tatistical Analysis:

The results were subjected to statistical analysis by applying student's t-test for ie determination of significance of difference between the body weights, testicular size nd testosterone levels of first half and last half of treatment period. Coefficient of orrelation between weekly body weights and testicular diameters was also calculated.

RESULTS

General observations

All the experimental animals were healthy and active at the start of experiment, and freely took food from the animal caretakers without showing any signs of pathological symptoms. However, following treatment with Aroclor 1254 and Aroclor 1242, they frequently developed pathological symptoms including the loss of appetite, diarrhea, lethargy, fever and vomiting during the treatment period. The general medicines including antibiotics as prescribed by the authorized Veterinary Officer were effective in overcoming these symptoms. However, the frequency of these pathological symptoms in PCB-treated animals increased with the passage of treatment period.

The experimental animals were also kept under regular observations to note the clinical signs of toxicity in all the external body parts. The animals in both the treated groups suffered from swelling of eyelids, edema under the eyes and showed signs of dermatological toxicity in the form of hair loss and appearance of lesions in nail-beds in the later part of treatment period. Though these clinical symptoms were observed in all PCB-treated animals, the severity was quite variable. The vehicle-treated animals remained free of such pathological disorders, excepting one animal, which showed some signs of hair loss.

The animals from both the PCB-treated groups, also exhibited some neurobehavioral changes in the later part of the treatment period. One animal of the Aroclor 1254-treated group, exhibited signs of distress by holding his head between the hands quite frequently. Two monkeys of this group became extraordinarily lethargic. Two animals from Aroclor 1242-treated group exhibited rash responses and frequently displayed signs of anger on seeing any person, including the caretaker while taking rest for most of the time. The vehicle-treated animals did not show such behavioral changes.

Effect on body weight

Mean body weight of Aroclor 1254-treated animals decreased significantly ($P<0.05$) at the end of treatment period as compared to the vehicle-treated group (Fig. 2). Decline in the body weights in Aroclor 1254-treated group was consistent in all animals.

Aroclor 1242-treatment slightly reduced body weights of the treated animals. However, there was no significant decrease in the body weights of both treated and control groups (Fig. 3).

Effect on testicular diameter

Testicular diameter of Aroclor 1254-treated animals also decreased gradually and significantly ($P<0.05$) during the treatment period. In contrast, vehicle-treated animals experienced gradual increase in testicular diameter with the passage of time (Fig. 4).

Testicular diameter of Aroclor 1242-treated animals also decreased significantly ($P<0.05$) during the experimental period as compared to vehicle-treated animals (Fig. 5).

The changes in body weight and testicular diameter were highly correlated (cf; 0.860767) in Aroclor 1254-treated animals. However, the correlation coefficient between body weight and testicular diameter in Aroclor 1242-treated animals was 0.114229 that was comparable to the correlation coefficient (0.19449) between body weight and testicular diameter in vehicle-treated animals.

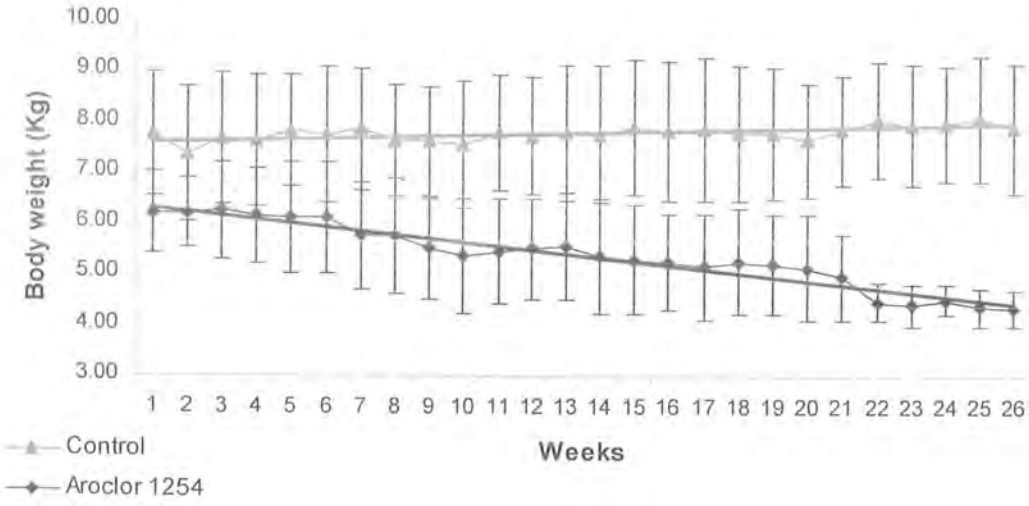


Fig. 2. Mean weekly body weights of Aroclor 1254-treated and vehicle treated animals. Vertical bars indicate SEM

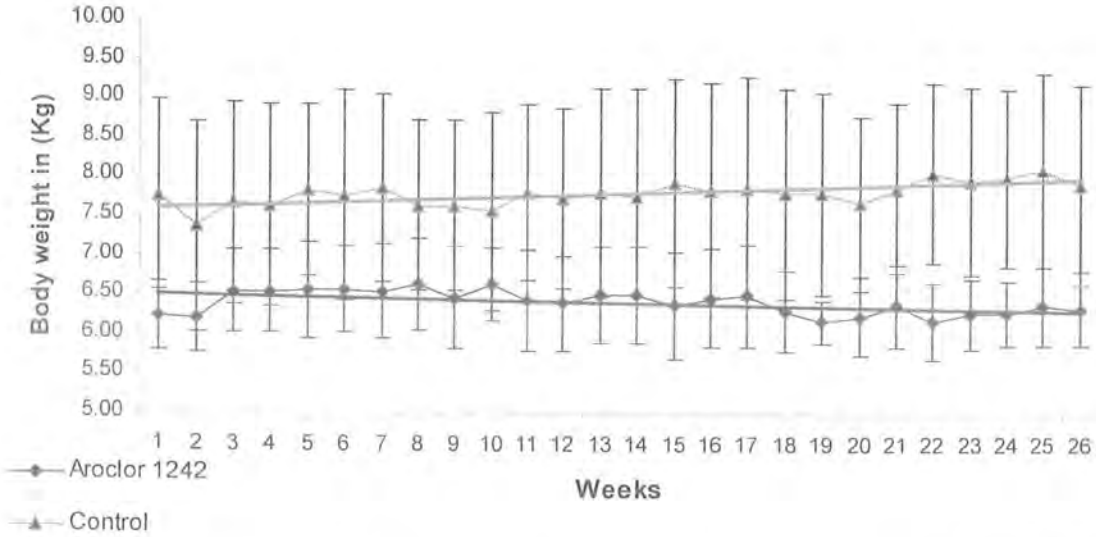


Fig. 3. Mean weekly body weights of Aroclor 1242-treated and vehicle treated animals. Vertical bars indicate SEM

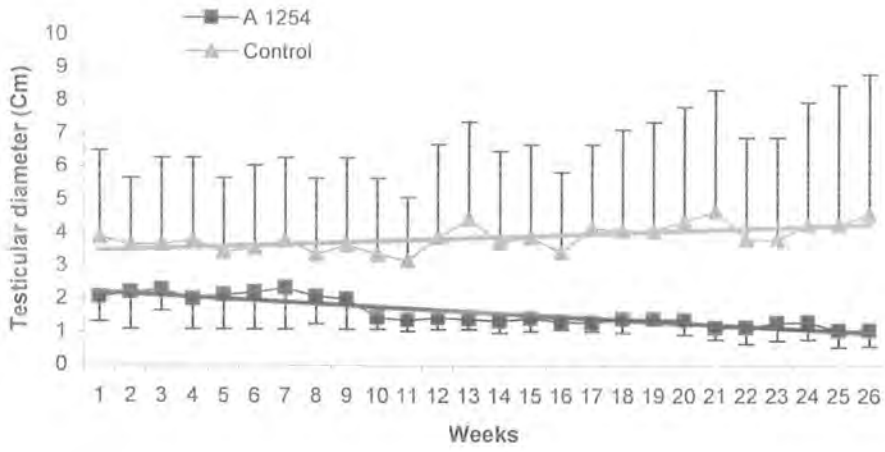


Fig. 4. Mean testicular diameter of Aroclor 1254-treated and vehicle treated animals. Vertical bars indicate SEM

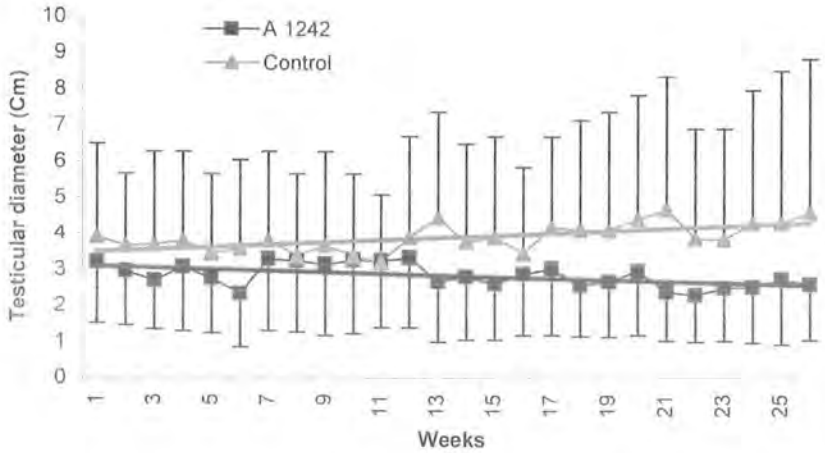


Fig. 5. Mean testicular diameter of Aroclor 1242 treated and vehicle treated animals. Vertical bars indicate SEM

Effect on plasma testosterone levels

Testosterone levels in the four Aroclor 1254-treated animals are shown in Figure 7 (a-d). Aroclor 1254-treatment did not affect the mean testosterone levels in the plasma throughout the treatment period (Fig. 6). There was no significant difference in the testosterone levels of Aroclor 1254-treated animals when the plasma samples of earlier half and late half of treatment period were compared. A similar trend was observed in vehicle-treated group.

Testosterone levels of Aroclor 1242-treated monkeys are presented in Figure 8 (a-d). Generally the mean testosterone levels of Aroclor 1242-treated animals declined during the treatment period (Fig. 8). However, testosterone levels of two members of the Aroclor 1242-treated group decreased significantly ($P<0.05$), whereas those of the other two did not change significantly when the plasma samples of earlier half and late half of treatment period were compared. The testosterone levels of the vehicle-treated animals remained unchanged.

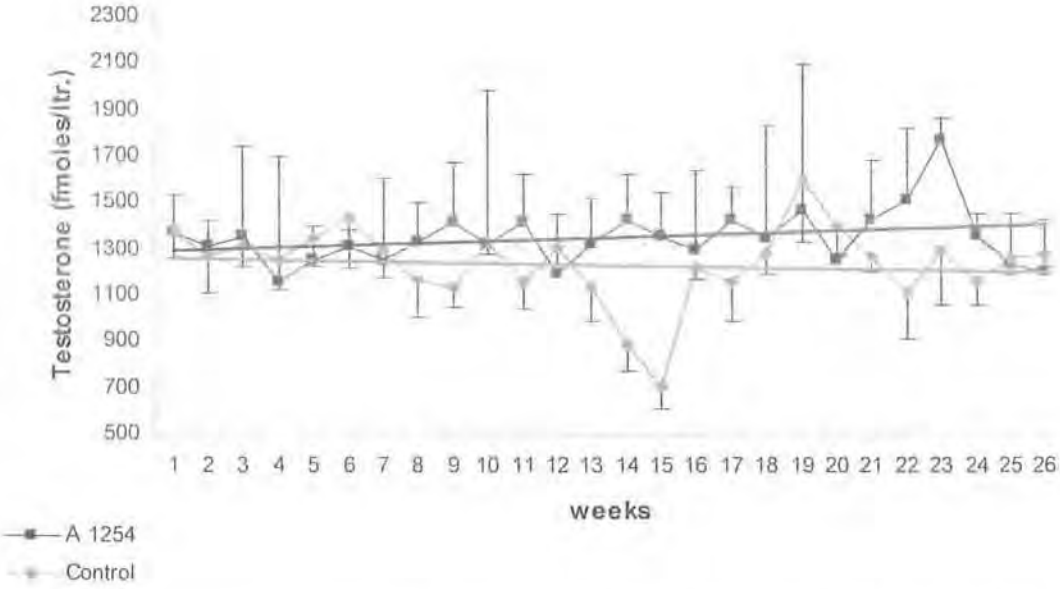


Fig. 6. Mean plasma testosterone levels in Aroclor 1254-treated and control animals during the treatment period. Vertical bars indicate SEM

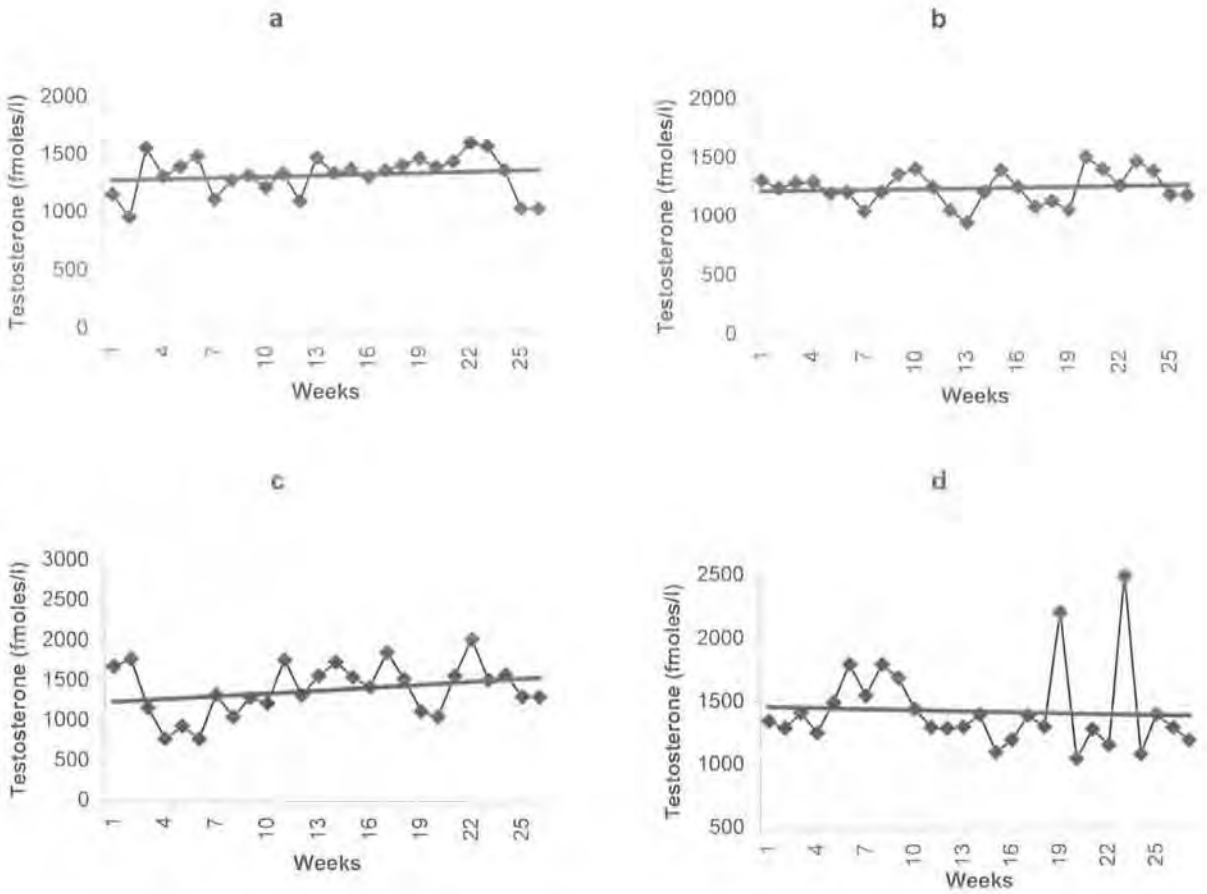


Fig. 7 (a-d). Plasma testosterone levels in the four Aroclor 1254-treated animals during the treatment period. Vertical bars indicate SEM

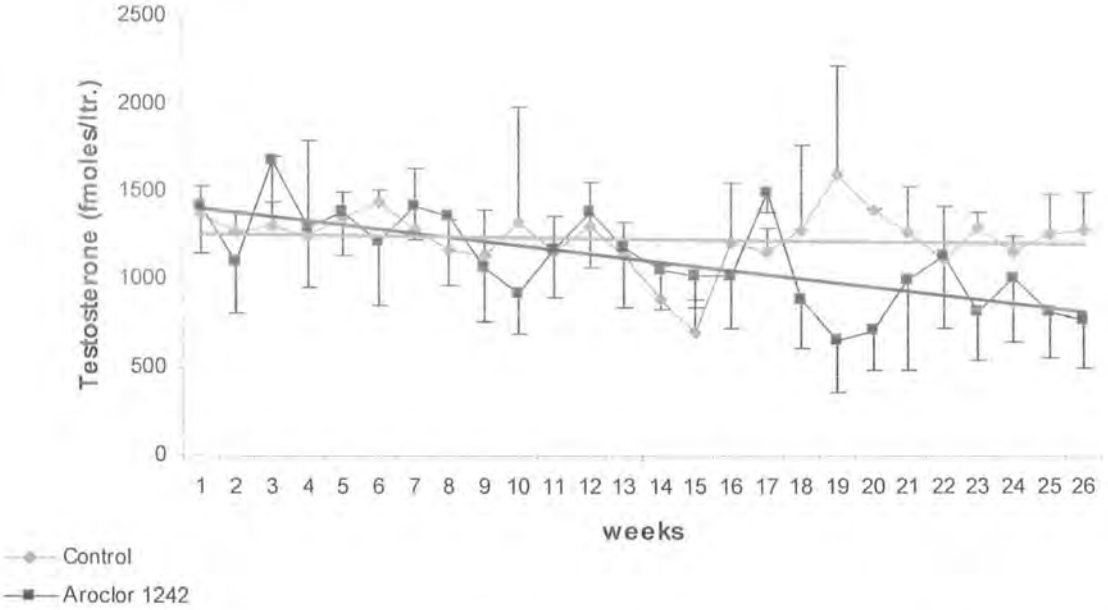
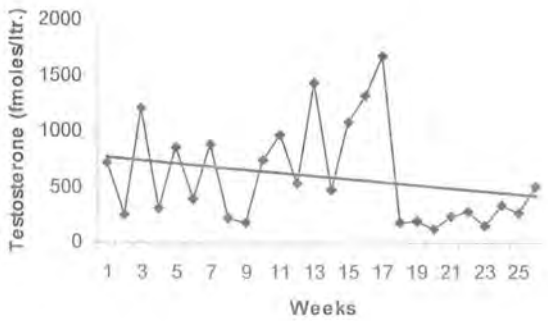
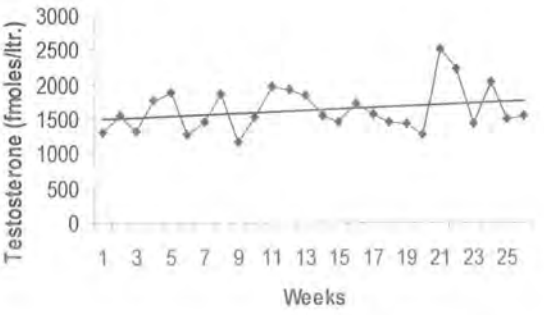


Fig. 8. Mean plasma testosterone levels in Aroclor 1242-treated and control animals during the treatment period. Vertical bars indicate SEM



a



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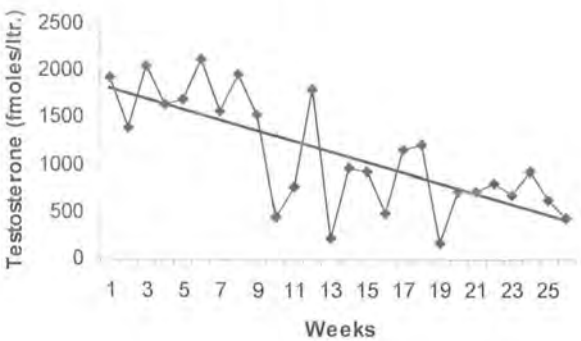
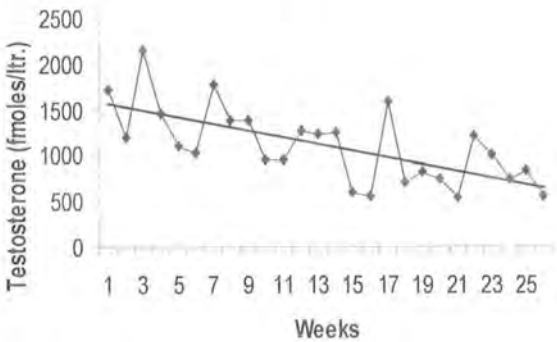


c



d

Fig. 9a-d. Plasma testosterone levels in the Aroclor 1242-treated animals during the treatment period. Vertical bars indicate SEM

Effect of Aroclor 1254 and Aroclor 1242 on the Morphology of testes

Aroclor 1254 and Aroclor 1242 given orally at a dose of 200 μg/Kg bw/day for 6 months caused marked changes in the histological and ultrastructural features of the testes.

***Light microscopic observations*:**

The seminiferous cords of the vehicle-treated monkeys exhibited typical arrangement of epithelial components. Among the spermatogonia, sub-types A (both *Ap* with pale nucleoplasm and *Ad* with darkly stained nucleoplasm) and B with patchy chromatin could be recognized. The spermatocytes had chromosomes and at many places lacked nuclear membranes. Spermatids, sperm, Sertoli cells in the seminiferous cords and Leydig cells in the interstitial spaces also visible and exhibited their normal features (Fig 10a &b).

Aroclor 1254- and Aroclor 1242-treatment caused much damage to the testes that resulted in disruption of normal epithelial organization, reduction/elimination of spermatozoa, spermatids, spermatogonia, shrinkage of Sertoli cells and delamination of the tunica propria. The overall cellular contents of the germinal epithelium was drastically reduced which made it possible to count the remaining cells in the seminiferous cords. A comparative analysis of various types of cells in the seminiferous cords of Aroclor 1254- and Aroclor 1242-treated monkeys is presented in Table 2. The diameter of the seminiferous cords ranged between 60-105 μm, 35-65 μm and 110-125 μm in Aroclor 1254-, Aroclor 1242- and vehicle-treated animals respectively (Table 3).

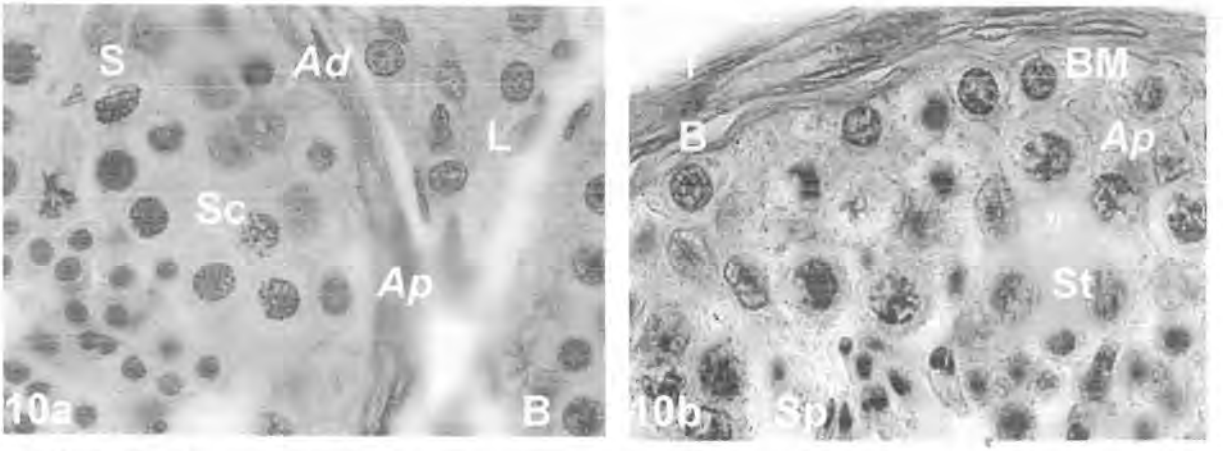


Fig. 10a &b. Photomicrographs of control monkey testes showing normal features of the seminiferous tubules X 40. (*Ad*: A dark type spermatogonium, *Ap*: A pale type spermatogonium, B; B type spermatogonium, BM: Basement membrane, S; Sertoli cell, Sc: Spermatocyte, Sp; Sperm, St: Spermatid, T; tunica propria)

Table 2

Mean ± SEM of major types of cells in the germinal epithelium of Aroclor 1254- and Aroclor 1242-treated monkey testes.

Animal	Spermatogonia		Spermatocytes		Spermatids		Spermatozoa		Sertoli	
	A 1254	A 1242	A 1254	A 1242	A 1254	A 1242	A 1254	A 1242	A 1254	A 1242
1	7±1.6	9± 1.7	11± 1.7	11± 3.1	9±1.3	8± 2.7	5± 1.3	14 ± 1.9	27± 4.6	57±11.1
2	8±1.7	12± 2.1	7± 1.4	27± 4.	7±1.6	31± 3.1	3± 1.7	34± 2.1	24± 3.7	66±9.7
3	5± 1.3	21± 2.4	5± 1.7	31± 2.1	6±1.3	47± 3.2	3±1.4	53 ± 3.5	24±6.4	106±19.4
4	6±1.1	17± 2.7	9± 1.2	23 ± 2.7	6± 1.7	37± 3.7	2± 1.3	43± 3.1	20± 6.3	79± 15.3

Table 3

Mean ± SEM of Diameter of seminiferous cords and spermatogonial size of the vehicle-, Aroclor 1254- and Aroclor 1242-treated animals

Animal	Seminiferous cord diameter (μm)			Spermatogonial size (μm)		
	Vehicle-treated	A 1254-treated	A 1242-treated	Vehicle treated*	A 1254 treated**	A 1242-treated***
1	123±27.3	105 ±18.4	35 ± 3.4	12 ±1.3	22 ±5.3	13 ± 2.3
2	125±22.1	73 ±16.7	47 ± 3.6	11 ±1.9	14 ±7.3	14 ± 1.9
3	115±17.3	60 ±13.4	65 ± 5.3	12 ±1.6	18 ±2.5	12 ± 2.6
4	119±11.7	87 ±19.7	53 ± 3.7	12 ±1.7	21 ±6.2	12 ± 2.7

** Significantly greater $P<0.05$ than * and ***

In the seminiferous tubules of Aroclor 1254-treated testes, the tunica propria appeared thicker than in the sections of vehicle-treated testes. It had wavy margins with indentations and the gap between the basement membrane and the peritubular Myeoid cell layer widened to a considerable extent. The Myeoid cells appeared necrotic with elongated, shrunken and hyperchromatic nuclei. The basement membrane of many tubules also appeared much swollen (Fig. 11a). In the testis of Aroclor 1242-treated animals too, the tunica propria disassembled at most of the places because of the disassembly of the tunica, the necrotic Myeoid cells with hyperchromatic nuclei were displaced towards the interstitium in many instances (Fig. 11b).

The seminiferous cords lacked the typical cellular arrangement of the germinal epithelium. In both of the Aroclor-treated groups, spermatogonia showed abnormalities in terms of number, size and cellular properties. The number of spermatogonia was far less in the treated animals than in their vehicle-treated counterparts (Table 2). The majority of the spermatogonia of the Aroclor 1254-treated animals were abnormally large in size with much variability in shape (Fig. 11 c & d; Table 3). In many instances, these cells contained empty spaces due to shrinkage of the cytoplasm, which also contained vesicles of various sizes. They rested on the basal part of the cords and were characterized by round to oval entirely euchromatic and hypertrophied nuclei. The average diameter of the nuclei of the spermatogonia was 7.2 μm. The cytoplasm of the spermatogonia was weakly stained. The average size of the spermatogonia of the Aroclor 1254-treated testes (18.75 ± 5.32) was significantly greater ($P<0.05$) as compared to those of the Aroclor 1242-treated (12.75 ± 2.375) and vehicle-treated testes (11.75 ± 1.625) (Table 3).

In the spermatogonia of Aroclor 1242-treated testes, such abnormalities in size and shape as observed in the testicular sections of the Aroclor 1254-treated animals were not evident. *Ap* and B type spermatogonia could be recognized in this group. *Ap* type spermatogonia were fewer containing round completely euchromatic nuclei. B type spermatogonia had round to oval nuclei with evenly dispersed patches of heterochromatin (Fig. 11b, e & f). Average nuclear diameter of the spermatogonia was 6.7μm.

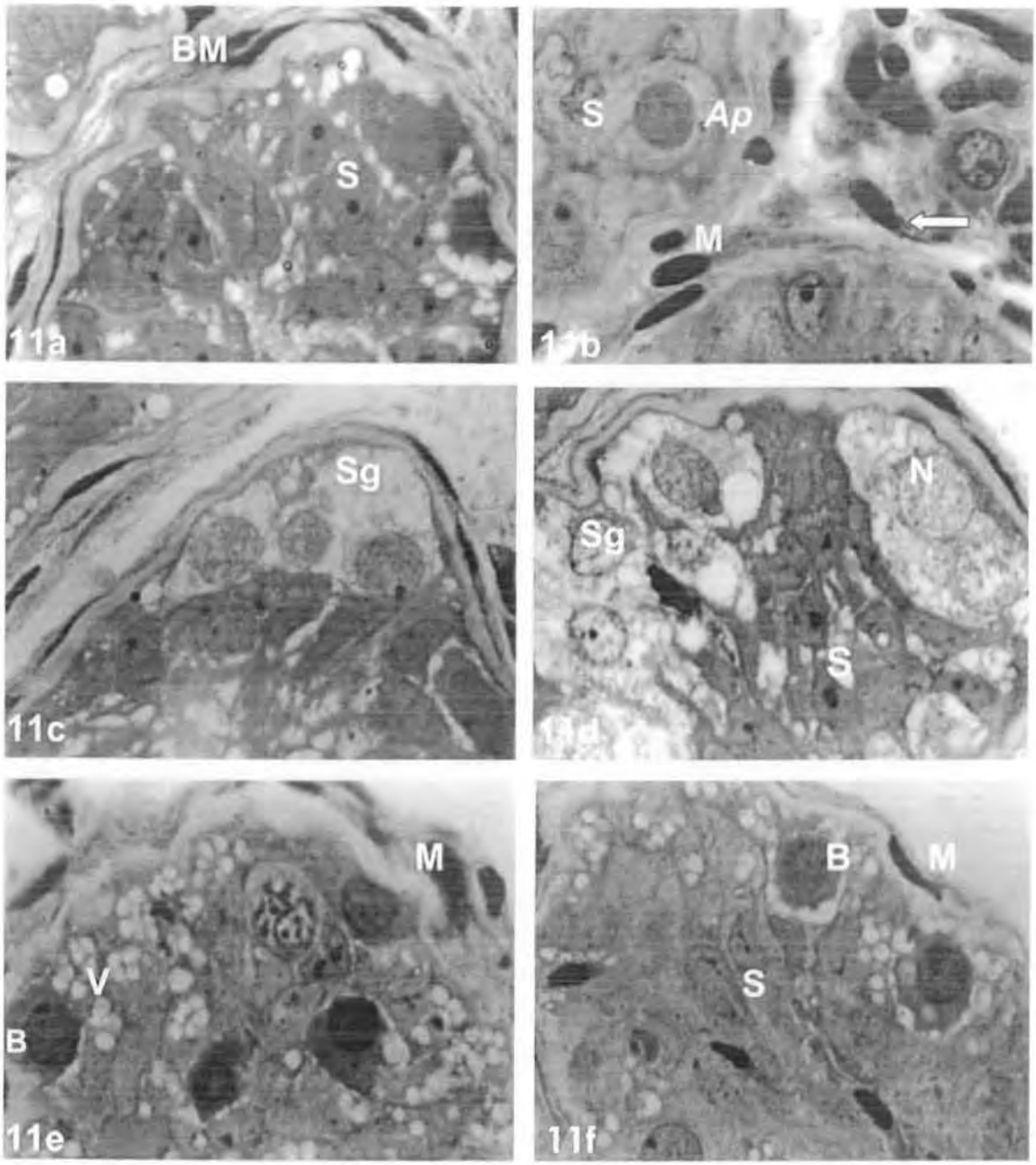


Fig. 11a-f. Photomicrographs of PCB-treated monkey testes showing the effects on seminiferous tubules. a. portion of Aroclor 1254-treated seminiferous tubule. Note that tunica propria has become much thicker X 40. b. the basement membrane has delaminated from the necrotic Myeoid cells penetrating interstitium (arrow) in Aroclor 1242-treated testis X 40. c & d. spermatogonia of Aroclor 1254-treated testes are abnormal in size and shape X 40 each. e & f. the spermatogonia of Aroclor 1242-treated testis exhibiting shrinkage and vesiculation in the cytoplasm X 40 each. (Ap; A pale spermatogonium, B; B type spermatogonium, BM; basement membrane, M; Myeoid cells, N; nucleus; S; Sertoli cells, Sg; Spermatogonium, V; vesicles)

The paucity of various spermatogenetic stages made Sertoli cells highly conspicuous which were also the most abundant cells in the testes of both treated groups (Table 2). They appeared shrunken in the sections of the Aroclor 1254-treated testes and occurred in clusters (Fig. 12a). Their typical irregular-shaped and indented nuclei were completely euchromatic and had prominent nucleoli. However, nuclear infoldings were reduced to a great extent (Fig. 12b).

The luminal areas in most of the sections of Aroclor 1242-treated testes were either occupied by the cytoplasmic extensions of the Sertoli cells (Fig. 12c) or by the debris of degenerating germ cells. The basal cytoplasm of the Sertoli cells contained numerous vesicles with a dense core (Fig. 12d).

The Leydig cells of the Aroclor 1254-treated and control (vehicle-treated) monkeys were more or less similar. The Leydig cells of the Aroclor 1254-treated monkeys were large and polyhedral having eccentric nuclei and granular cytoplasm with a lot of lipid droplets. These cells were scattered randomly in the interstitium (Fig. 12e). Their nuclei were round with prominent nucleoli and peripheral heterochromatin.

Leydig cells of Aroclor 1242-treated testes had round nuclei with variable hyperchromatic portions. However, some cells were highly distorted and contained pyknotic nuclei. Nuclei had mostly centrally placed nucleoli. Cytoplasm was scanty. At some places, the Leydig cells also contained fat droplets and showed vacuolation (Fig. 12f).

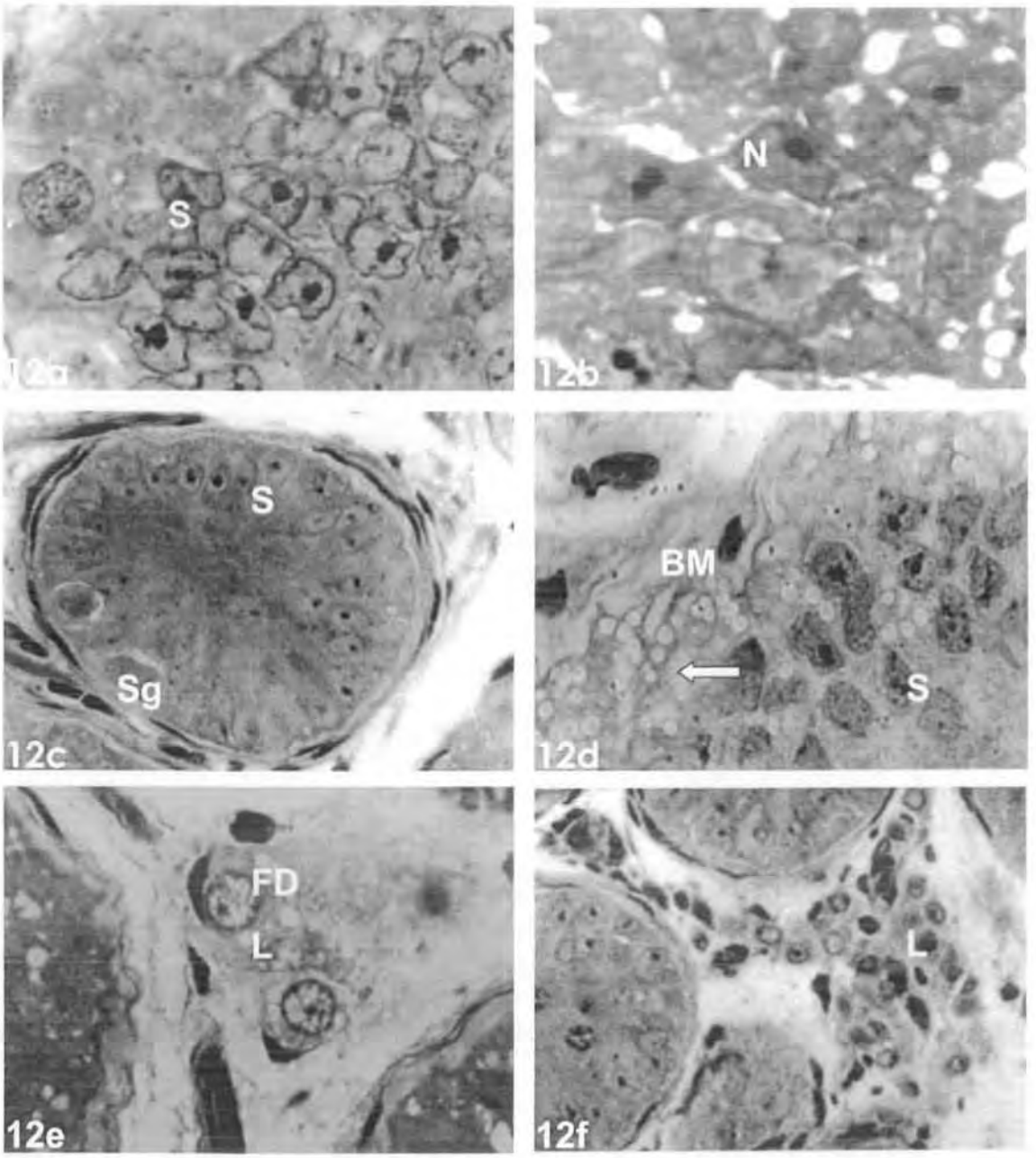


Fig. 12 a-f. Photomicrographs of PCB-treated monkey testes showing the effects of pollutant on various cell types. a & b. the Sertoli cells are crowded and shrunken in Aroclor 1254-treated testes X 40 each. c. In the Aroclor 1242-treated testes, the cytoplasmic extensions of Sertoli cells have even penetrated in the lumen and besides Sertoli cells, only two spermatogonia are present in the whole cord X 20. d. The Aroclor 1242-treated Sertoli cells contain a lot of vesicles towards the periphery (arrow) X 40. e. The Leydig cells of Aroclor 1254 treated testes appearing normal X 40. f. Necrosis is visible in the Leydig cells of Aroclor-1242-treated testes X 10. (BM; basement membrane, FD; fat droplets, L; Leydig cells, M; Myeoid cells, N; nucleus, S; Sertoli cell, Sg; spermatogonium).

Electron microscopic observations

In the vehicle-treated testes, the Myeoid cells maintained their position between the internal lamellae (basement membrane) and external lamellae which was supported by connective tissue fibers (Fig. 13). The ultrastructural features of both PCB-treated groups were more or less similar. The tunica propria was completely disorganized. The basement membrane dissociated itself from the Myeoid cells creating a wide space that was occupied by disassembled collagenous fibers. The Myeoid cells appeared necrotic containing indented nuclei with a prominent heterochromatic rim and nucleolus. They contained far less cytoplasm than in the cords of vehicle-treated group. The external lamella was not visible at many places. Due to the detachment of external lamella, the cell membrane of these cells at some places appeared hairy due to the remnants of collagenous fibers at the surface of cell membrane. The internal and external lamellae became wide apart and the former split into several layers (Fig. 14a-c).

Fig. 13. Electron micrograph of a control testis showing Myeoid cell sandwiched between internal lamella (basement membrane) and the external lamella supported by connective tissue X 20,000. (BM; basement membrane, CF; collagenous fibers, EL; External lamella, M; Myeoid cell, N; nucleus)

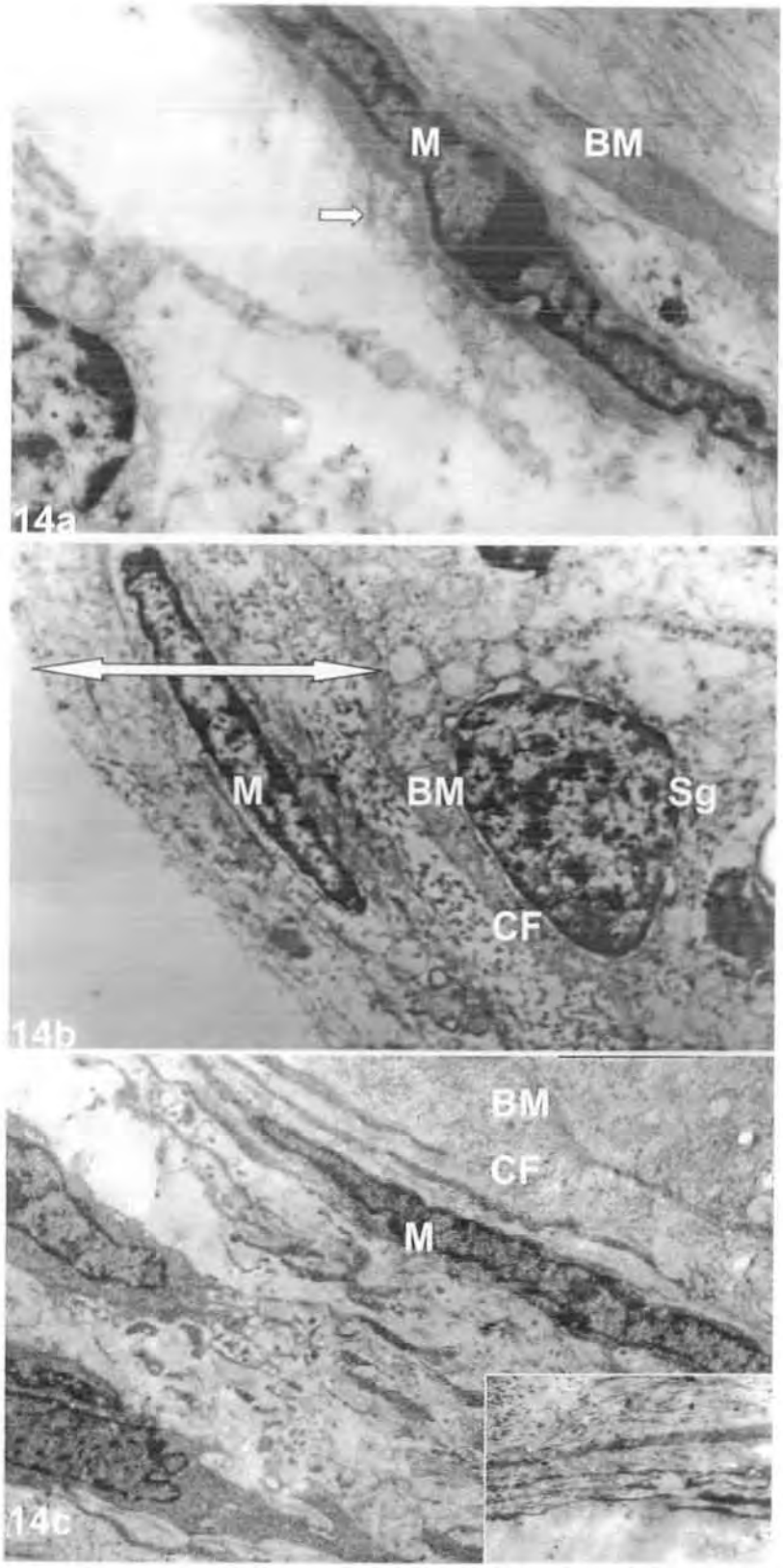


Fig. 14a-c. Electron micrographs of Tunica propria of PCB-treated monkey testes. a. Note the detachment of basement membrane and external lamella from Myeoid cell in Aroclor 1254-treated testis and hairy appearance of cell membrane of Myeoid cell (arrow) X 7000. b. In the Aroclor 1242-treated testes, wider gap between the lamellae (double arrowhead) and disassembly of collagenous fibers (b X 5000) and sub-layering of basement membrane (box) is evident (c X 15000). (BM; basement membrane, CF; collagenous fibers, M; Myeoid cells).

The spermatogonia of vehicle-treated monkey testes had round or oval nuclei with a variable distribution of heterochromatin. In some nuclei, heterochromatin was in the form of dense bodies usually associated with nuclear membrane. Mitochondria and smooth endoplasmic reticulum were conspicuous in the spermatogonia of vehicle-treated monkeys (Fig. 15a).

In the seminiferous cords of the Aroclor 1254-treated monkeys, differentiation between the various types of spermatogonia was difficult owing to their paucity and the damage caused by the pollutant in the cytoplasm and nucleus. In many cells, the nucleus lost its normal round/oval shape and seemed shrunken. The amount of heterochromatin also increased, and the cytoplasm developed clear patches. None of the organelles excepting mitochondria could be recognized in most of the sections. Vacuolation of the cytoplasm was evident in some cells (Fig. 15b).

In the spermatogonia of Aroclor 1242-treated testes, a number of abnormalities were observed. In A type spermatogonia, a lot of empty spaces developed due to shrinkage of the cytoplasm. The cells contained only mitochondria, which were often clustered. However, the cell membrane was intact and in contact with surrounding Sertoli cells (Fig. 16a). In B type spermatogonia, nuclei were round/ oval and contained patches of heterochromatin. Mitochondrial cristae appeared distorted and the endoplasmic reticulum was rare. The nuclei too lost their round or oval shape owing to shrinkage and in many instances the nuclei was highly pyknotic with more than normal heterochromatic portions. The cells were highly shrunken with disruption of the cell membrane at some places. Although, the cell membrane of spermatogonia was in contact with the adjacent Sertoli cells, junctional complexes were not seen (Fig. 16b).

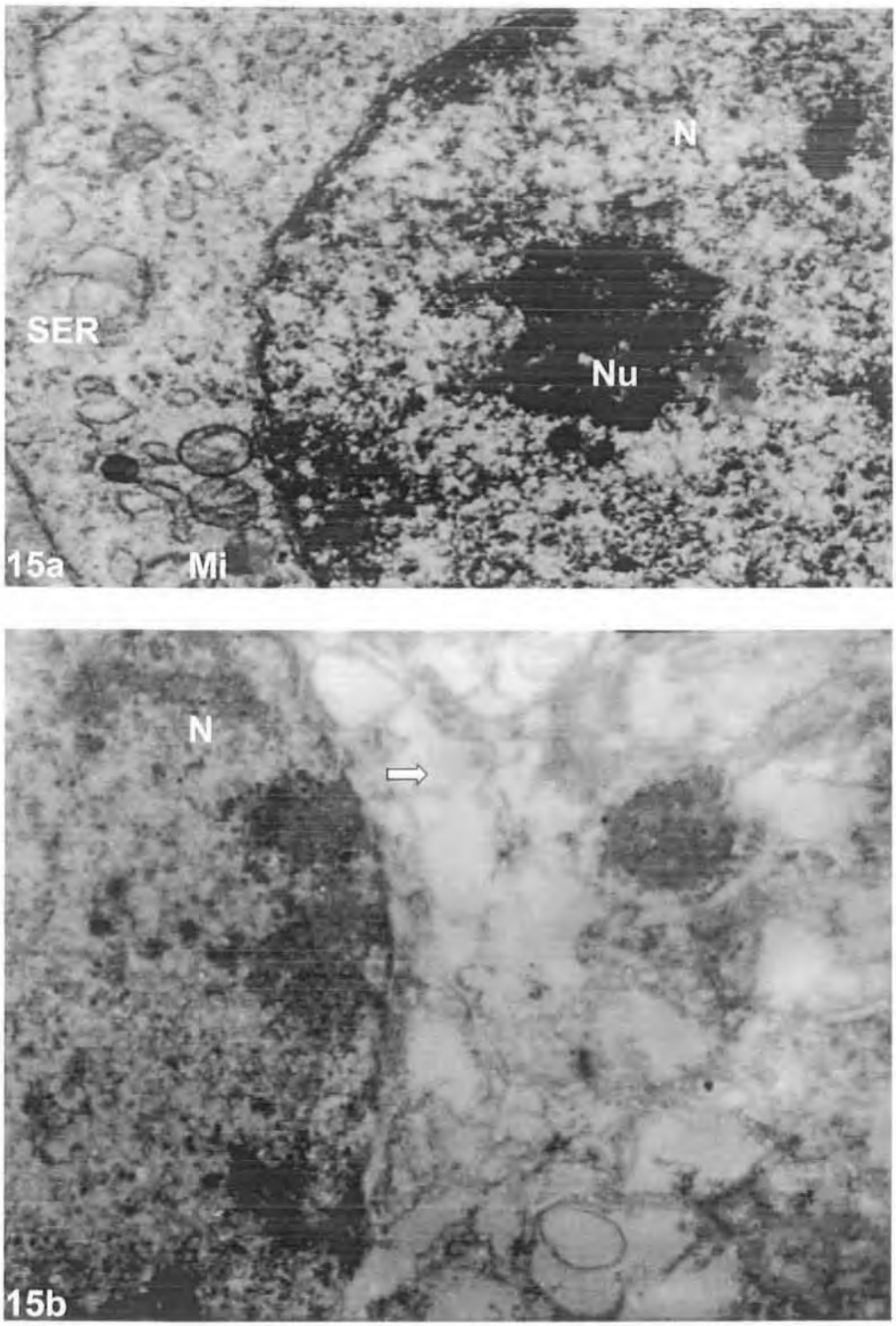


Fig. 15a & b. Electron micrographs of spermatogonia. a. A spermatogonium of vehicle-treated testis X 15000. b. A spermatogonium of Aroclor 1254-treated testes exhibiting disorganization of cytoplasm with prominent vacuolation (arrow) and nucleus with more heterochromatin X 15000. (Mi: mitochondria, N; nucleus, SER; smooth endoplasmic reticulum).

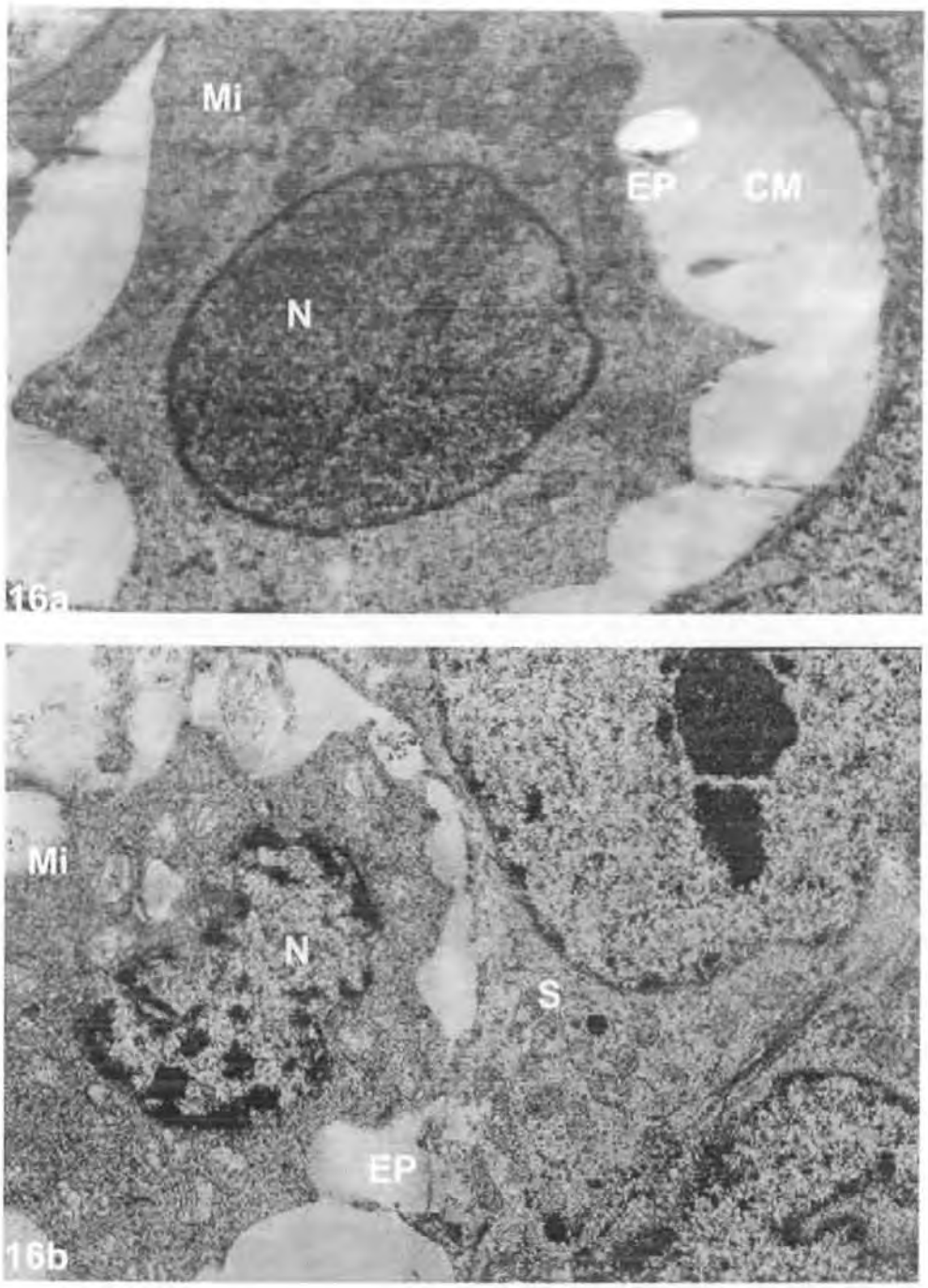


Fig. 16a & b. Electron micrographs of Aroclor 1242-treated testes. a. Extreme shrinkage of cytoplasm in spite of intact cell membrane. Nucleus is entirely euchromatic X 10000. b. Spermatogonium possess highly pyknotic nucleus and distorted mitochondria besides exhibiting shrinkage of the cytoplasm X 8000. (CM: cell membrane, EP: empty space, Mi: mitochondria, N: nucleus, S: Sertoli cell).

The spermatocytes of the vehicle-treated testes showed various stages of meiosis. At some places, the nuclei were round with dense chromatin and at other places, they were characterized by the condensation of chromatin where the nucleoli lost their identity. At some places, loosely coiled nucleoli with poorly granulated nucleonema could be recognized. In some cells, the nuclear membrane was not visible and the chromatin condensed as chromosomes (Fig. 17a & b). In the vehicle-treated testes, the process of spermeogenesis was evident by the presence both round and elongated spermatids having completely euchromatic nuclei, acrosomal formation and presence a lot of spermatozoa in the lumen of seminiferous cords (Fig. 17c-h).

In the seminiferous tubules of Aroclor 1254-treated testes, the spermatocytes were rare and highly necrotic with patchy heterochromatin and vacuolated cytoplasm (Fig. 18a). In the Aroclor 1242-treated testes, some of the spermatocytes were active as evidenced by the presence of condensed chromatin. However, the degree of chromatin condensation was lower than in the vehicle-treated spermatocytes. Also, unlike the spermatocytes of the vehicle-treated testes, they had well developed nucleoli. The cytoplasm of most of cells was shrunken (Fig. 18b).

While the process of spermeogenesis was not evident in the Aroclor 1254-treated testes, it was adversely affected in Aroclor 1242-treated testes. The damage was characterized by shrinkage of the cytoplasm and thickening of cell membrane of the round spermatids (Fig. 18c), distortion of the elongated spermatids (Fig. 18d) and absence of stages of acrosomal formation. Though a few sperms were found, these were abnormal showing shrunken heads and absence or degeneration of tails (Fig. 18e & f).

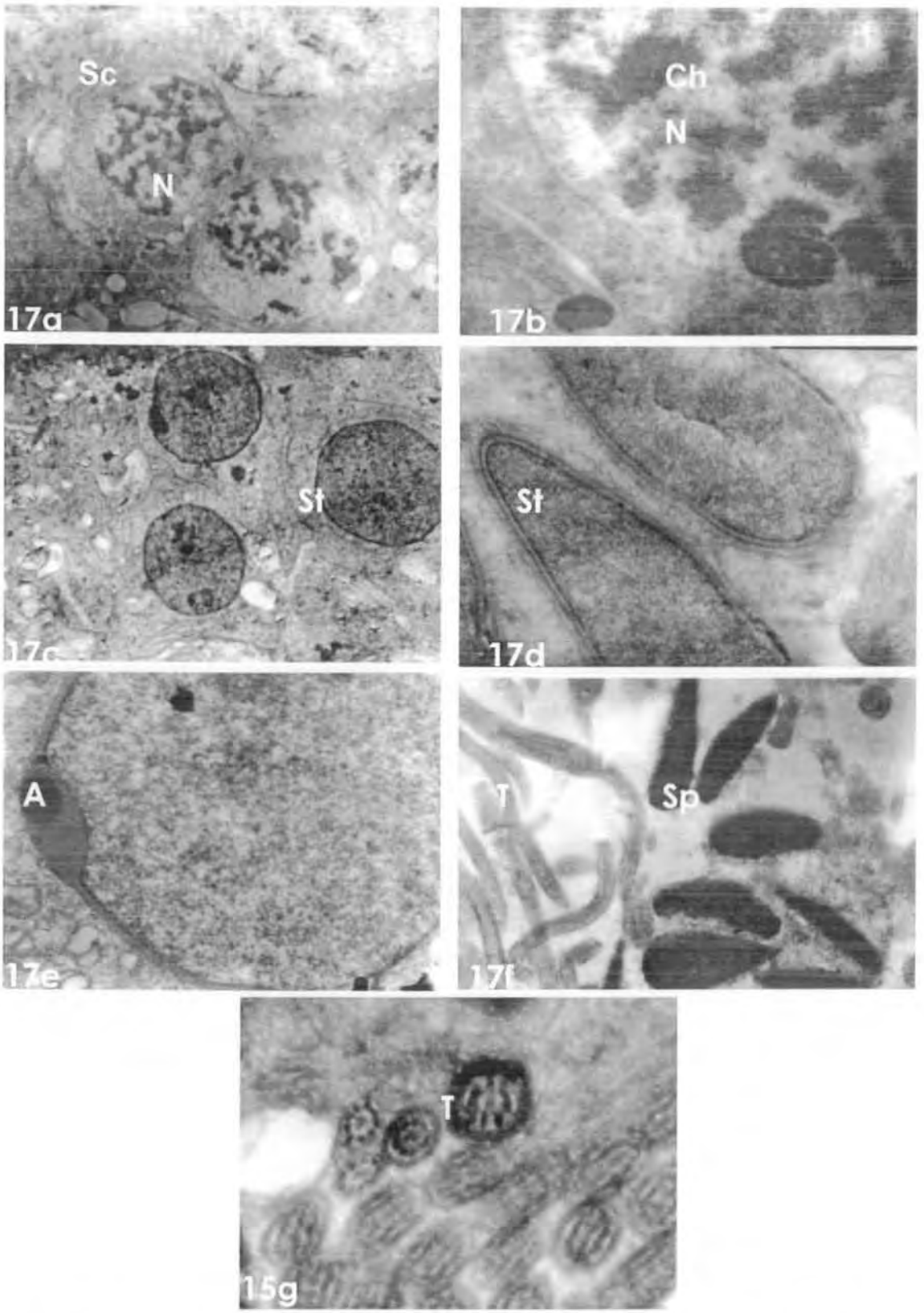


Fig. 15e-f. Electron micrographs of vehicle-treated testes showing spermatocytes and various features of spermeogenesis. a & b. Spermatocytes exhibiting chromosomes (a) X 3000 & (b) 8000. c & d. round and elongated spermatids respectively (c) X 6000 & (d) X 10000. e. Acrosomal formation stage X 10000. f. Spermatozoa X 8000. g. cross and longitudinal sections of the sperm-tails X 20000. (Ch: chromatin, N: Nucleus, Nu: nucleolus, Sc: spermatocyte, T: tail)

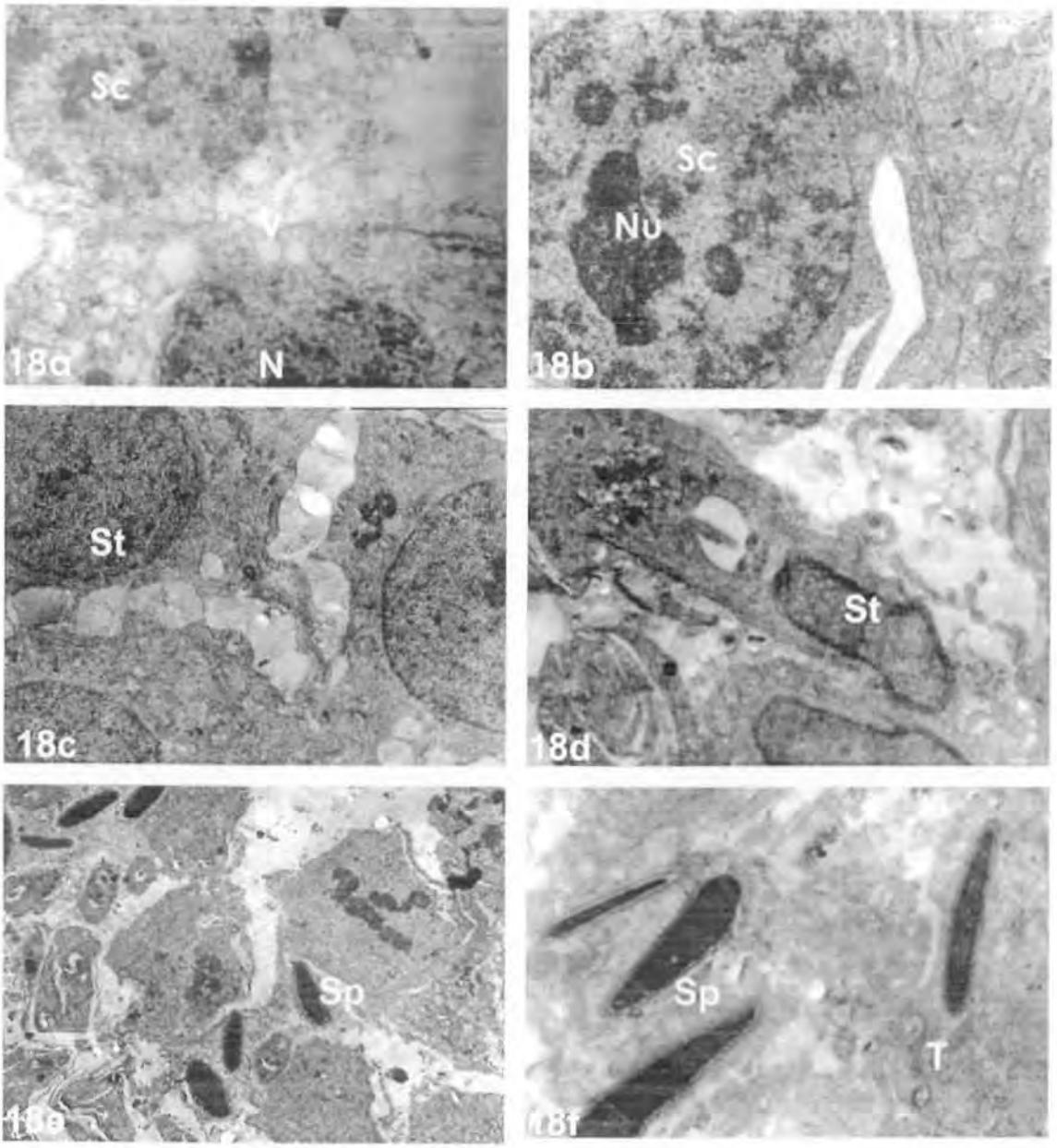


Fig. 18a-f. Electron micrographs of Aroclor PCB-treated monkey testes showing the effects of pollutant on the spermatocytes and spermeogenesis. a. spermatocytes of Aroclor 1254-treated testis exhibiting a lot of vacuolation and low degree of chromatin condensation X 5000. b. a spermatocyte of Aroclor 1242-treated testis having empty space within cytoplasm, low degree of chromatin condensation and a prominent nucleolus X 8000. c. round spermatids containing shrinkage in the cytoplasm X7000. d. distortion in the shape of elongated spermatids X 4000. e & f. spermatozoa heads exhibiting variation in size and shape and degeneration of fibers within tails (e) X 3000 & (b) X 8000. (Nu; nucleolus, N; nucleus, Sc; Spermatocyte, Sp: Sperm, St: Spermatid, T: tail, V; vacuolation).

In the vehicle-treated testes, the Sertoli cells had large and ovoid nucleus with characteristic infoldings. The nucleoplasm was homogeneous with one or more prominent nucleoli (Fig. 19a). The cytoplasm was electron pale with pronounced smooth and rough endoplasmic reticulum, mitochondria, lipid droplets (Fig. 19 b), well-developed Golgi complex (Fig. 19a) and varying number of microtubules, vesicles and filaments. The unique ectoplasmic specializations of Sertoli cells including the narrow intercellular space, condensation of filaments in the cytoplasm adjacent to the apposed cell membranes were also visible.

In the testes of both the PCB-treated groups, the Sertoli cells were much reduced in size and lacked the normal organization of organelles. Nuclear shape varied considerably and the infoldings of the nuclei were also reduced to a great extent as compared to those in the vehicle-treated nuclei of Sertoli cells and heterochromatin portion of the nucleoplasm was increased. In Aroclor 1254-treated testes, rarely seen ectoplasmic specializations of the Sertoli cells, were also damaged. Mitochondria were numerous. The cell membrane was disrupted at many places and the cytoplasm contained empty spaces (Figs. 20a & b). The Sertoli cells, the major components of the seminiferous cords of Aroclor 1242-terated testes, were also not normal. Shrinkage was visible in the cytoplasm due to which at many places, and the cytoplasm contained empty spaces of variable sizes. Mitochondria, endoplasmic reticulum, Golgi apparatus and fat droplets were quite conspicuous (Fig. 21a &b).

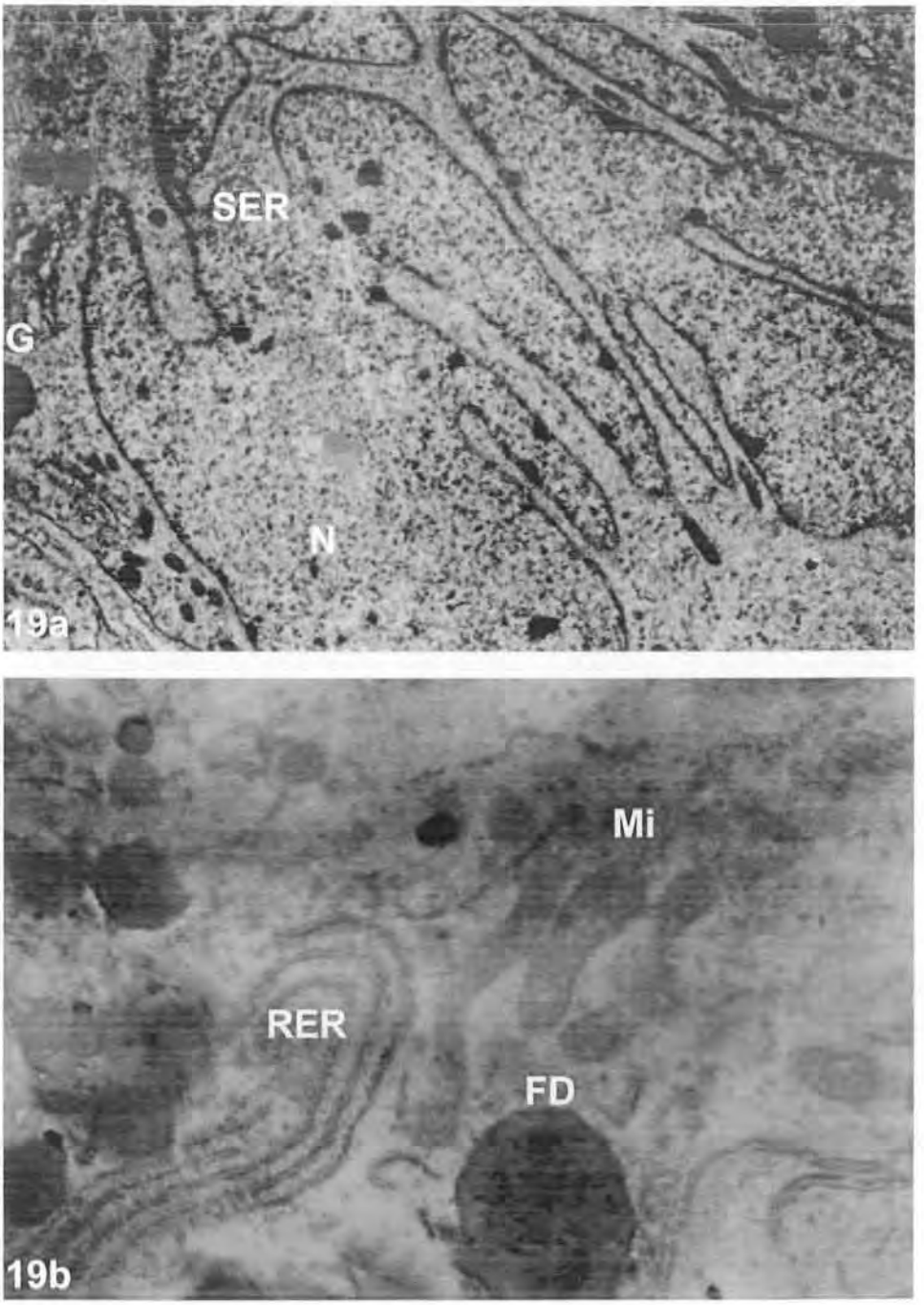


Fig. 19a & b. Electron micrographs of vehicle-treated testes showing the features of Sertoli cells. a . characteristic nucleus with cytoplasm penetrating within the infoldings X 12000. b. Cytoplasm containing organelles X 17000. (RER; rough endoplasmic reticulum, SER; smooth endoplasmic reticulum, FD; fat droplets, Mi; mitochondria, N; nucleus).

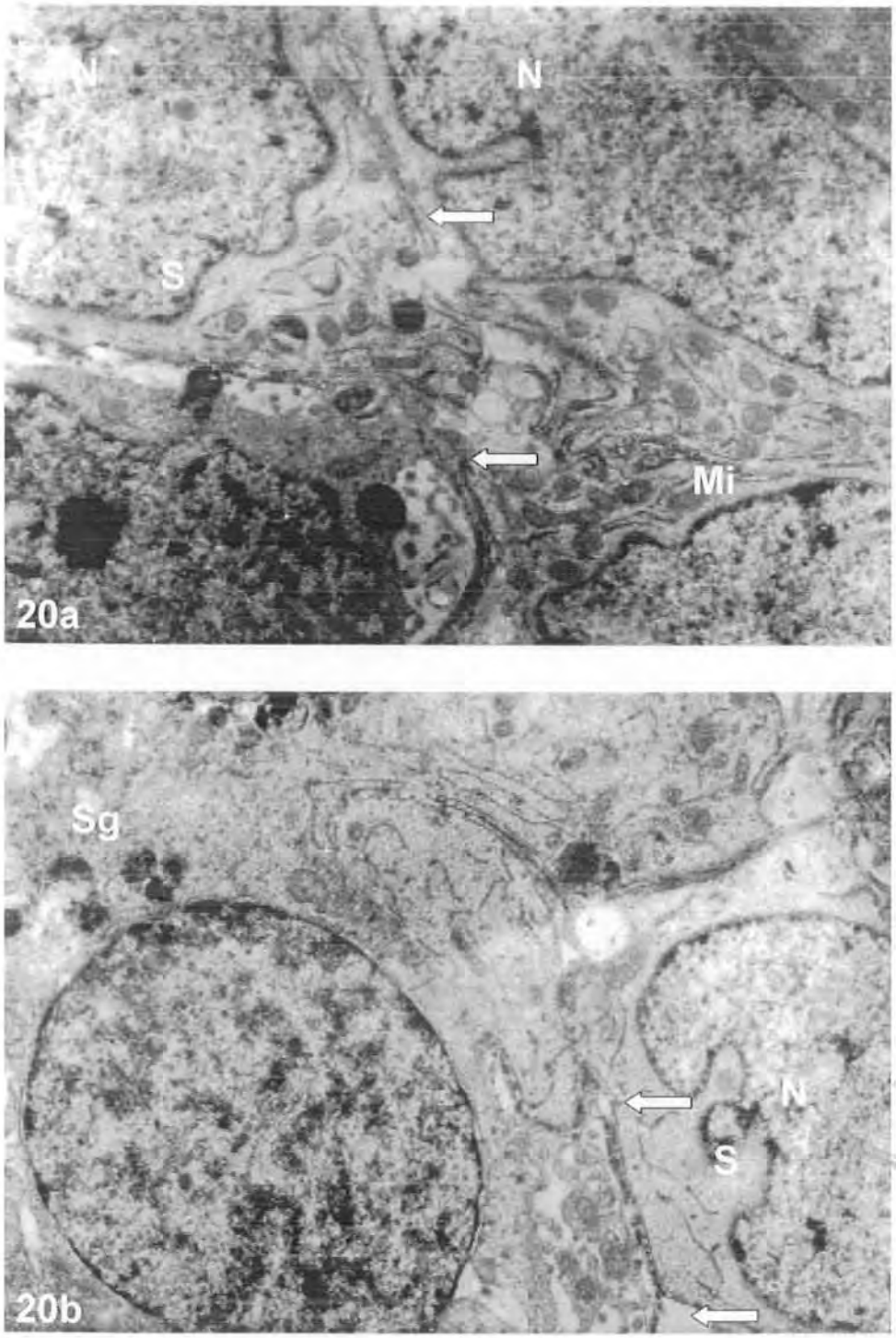


Fig. 20a & b. Electron micrographs of Aroclor 1254-treated testes showing the effect of pollutant on Sertoli cells. Note that the nuclei have fewer infoldings and cytoplasm is exhibiting high degree of disorganization. Ectoplasmic specializations are damaged (arrows) (a) X 6000 & (b) X 7000. (Mi; mitochondria, N; nucleus, S; Sertoli cell, Sg; spermatogonium).

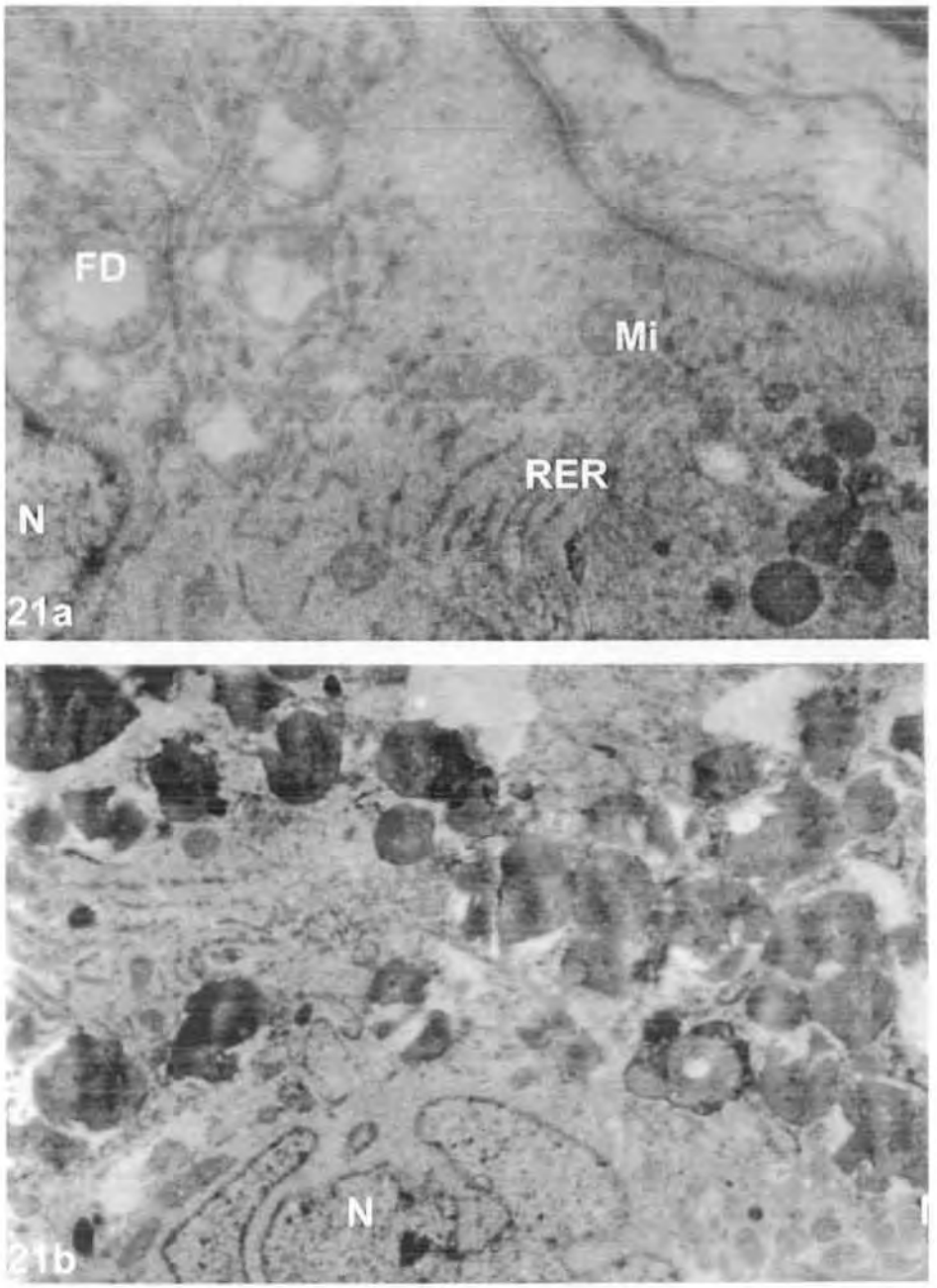


Fig. 21a & b. Electron micrographs of Aroclor 1242-treated testes showing the effect of pollutant on the Sertoli cells. Note that fat droplets are occupying most of the cytoplasm that also contain mitochondria and rough endoplasmic reticulum (a) X 15000 & (b) X 8000. (FD; fat droplets, RER; rough endoplasmic reticulum, Mi; mitochondria, N; nucleus).

In the vehicle-treated testes, the Leydig cells appeared large with a single large eccentric nucleolus in the nucleus. A heterochromatin rim was observed adjacent to the nuclear membrane. The cytoplasm was rich in organelles, of which the SER and mitochondria were very abundant (Fig. 22a).

In Aroclor 1254-treated testes, the Leydig cells contained round to oval nuclei having variable amounts of heterochromatin mostly in the form of a peripheral rim. The cytoplasm contained abundant fat droplets, SER and mitochondria (Fig. 22b).

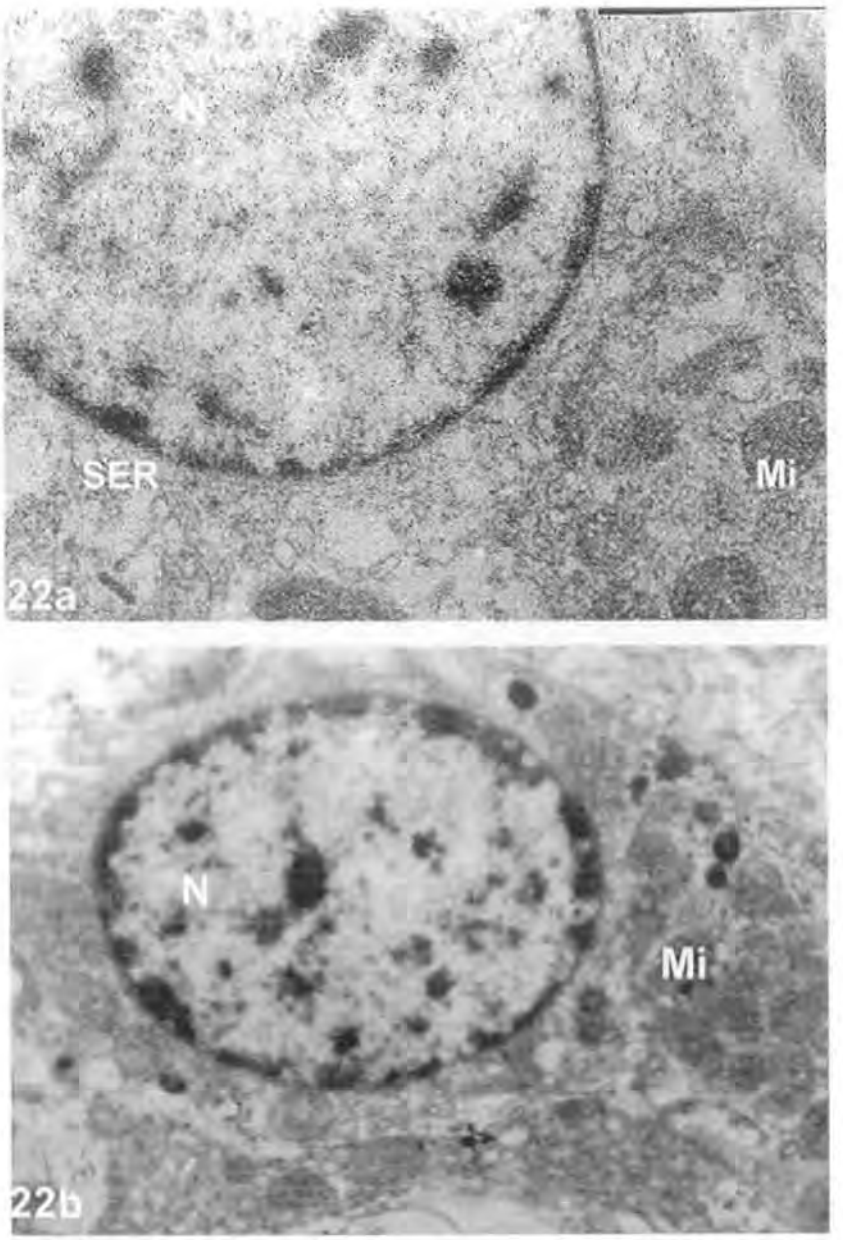


Fig. 22a & b. Electron micrographs of the testes showing Leydig cells. a. The Leydig cells of vehicle-treated testis having cytoplasm rich in SER and mitochondria X 8000. b. The Leydig cell of Aroclor 1254-treated testis exhibiting oval shaped nucleus with a heterochromatin rim and cytoplasm rich in mitochondria and SER X 5000. (FD; fat droplets, RER; rough endoplasmic reticulum, Mi; mitochondria, N; nucleus).

The Leydig cells of the Aroclor 1242-treated animals showed a number of abnormalities at both nuclear and cytoplasmic levels. Cytoplasm of these cells developed zones of electron dense and electron opaque regions. At some places, the cytoplasm was highly electron dense, appearing as a plaque with no evidence of organelles. This picture was in contrast to neighboring active cells, which had granular cytoplasm, a lot of mitochondria and a round nucleus (Fig. 23a). The cells with electron dense cytoplasm were heavily vesiculated and appeared in different stages of degeneration (Fig. 23b). The nucleus in many cells, lost its round or oval shape and became elongated or sickle shaped with variable degree of nuclear pyknosis (Fig. 24a &b). The heterochromatin was variably scattered all over the nucleoplasm with only a peripheral ring evident all round the inner side of the nuclear membrane. The cell membrane of several cells produced thread like extensions. Such cells contained very little cytoplasm (Fig. 24c).

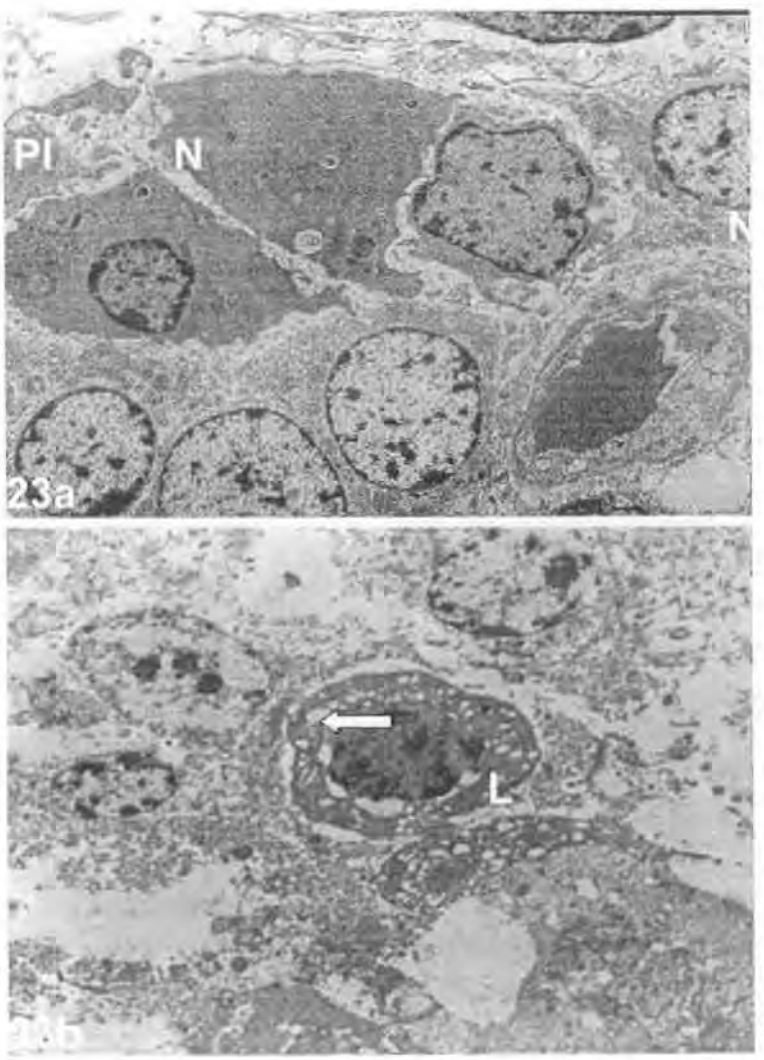


Fig. 24a & b. Electron micrographs of Aroclor 1242-treated testes showing the effect of pollutant on the Leydig cells. a. highly electron dense cytoplasm appearing as plaque in some cells while neighboring cells appearing normal X 4000. b. The cytoplasm contain clear patches in a cell while other appearing highly necrotic X 3000. (Pl; plaque, N; Nucleus).

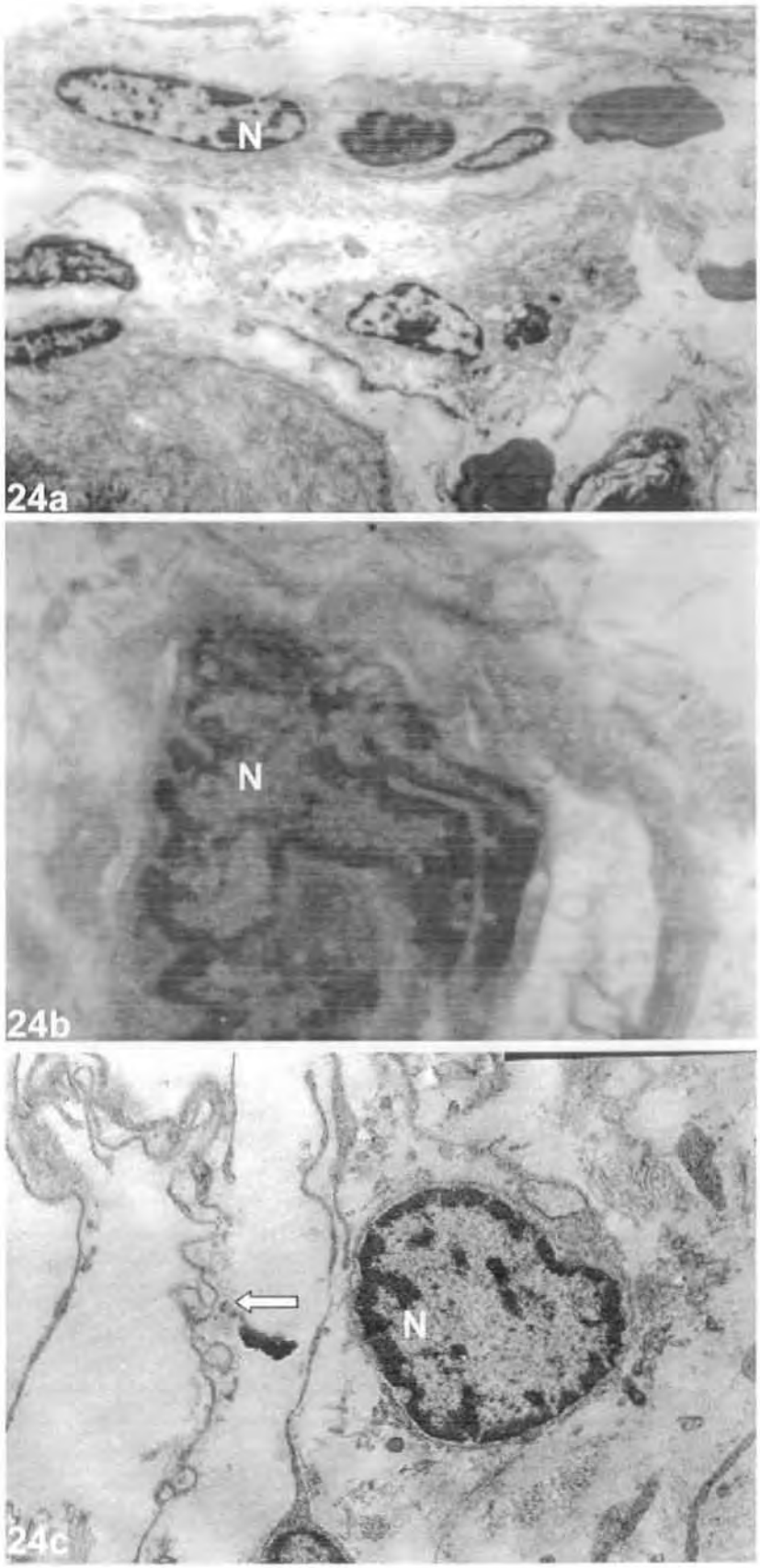


Fig. 24a-c. Electron micrographs of Aroclor 1242-treated testes showing the effect of pollutant on the Leydig cells. a & b. nuclear pyknosis and shape abnormalities (a) X 3000 & (b) X 8000. c. cytoplasmic abnormalities especially thread like projections (arrow) (c) X 5000 (N; nucleus).

Effect of Aroclor 1254 and Aroclor 1242 on the histology of accessory glands

All accessory glands were vulnerable to PCB treatment. The histological features of these organs qualitatively differed in the Aroclor 1254-treated and Aroclor 1242-treated animals from those of the control animals.

Epididymides:

The epididymides of the control monkeys were lined with pseudostratified columnar epithelium surrounded by a smooth muscle layer. The tall Principal cells could be distinguished from the basally located supporting cells. The Principal cells possessed stereocilia at their apical surface and oval nuclei placed basally close to the basal lamina. The nuclei of the supporting cells were more or less round and were also basal in position. The lumen of the gland contained a large number of sperms (Fig. 25a & b).

The histological picture of the epididymides of the Aroclor 1254-treated animals showed a sharp contrast to that of the vehicle-treated animals. The luminal spaces were much reduced in size and lacked spermatozoa. At some places, the lumen was completely obliterated. The epithelium of the organ in much thicker and stratified than in the controls. The cells were necrotic and lacked stereocilia. It was not possible to discriminate between the Principal and supporting cells owing to the adverse effects of the pollutant. The amount of connective tissue surrounding the gland also increased compared to its amount in the control epididymides (Fig. 25c & d). At some places the cells became abnormally large with the nucleus having a regular hyperchromatic ring, one or two nucleoli and clear cytoplasm

In the epididymides of Aroclor 1242-treated animals also, the luminal spaces lacked spermatozoa. The epithelium contained irregular shaped necrotic cells with stereocilia still visible. No distinction between the cell types was possible in the epithelial compartment due to disruption of its integrity. The amount of connective tissue was far greater as compared to that in the control epididymides (Fig. 25e & f).

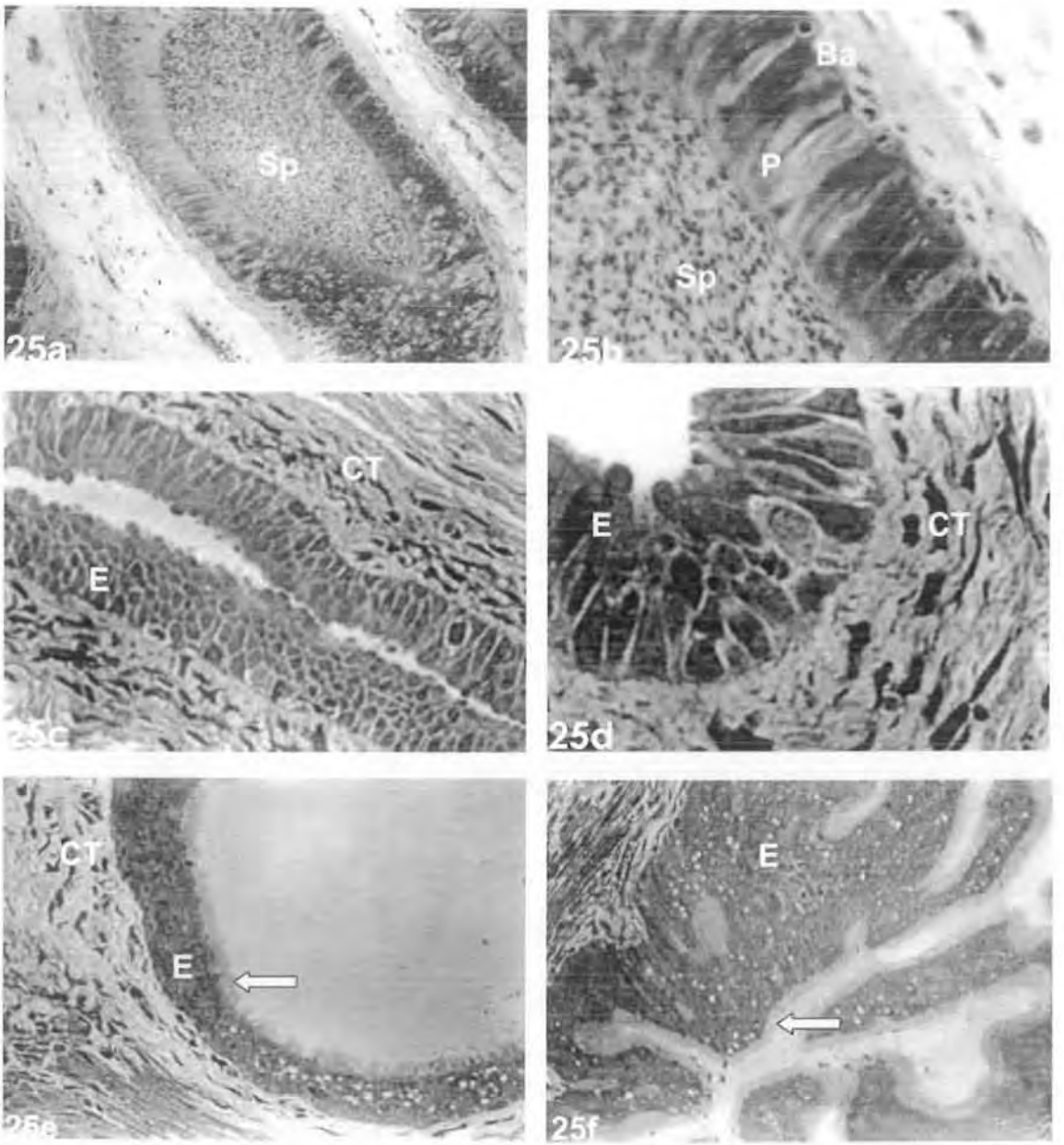


Fig. 25a & b. Photomicrographs of monkey epididymides. a & b. Vehicle-treated epididymis showing Principal cells and the basal cells in the epithelium surrounded by connective tissue. c & d Aroclor 1254-treated epididymis exhibiting collapsed lumen and stratification of epithelium where cellular distinction is not evident. e & f. Aroclor 1242-treated epididymis showing that the luminal margins of epithelium posses brush border (arrow) and the thickness has increased manifolds at some places. (E; epithelium, CT, connective tissue, Ba; basal cells, P; Principal cells, Sp; spermatozoa).

Prostate Gland:

In the acinitubular prostate glands of the vehicle-treated monkeys, the pseudostratified epithelium consisted of columnar or cuboidal cells and a few basal cells. The stroma was occupied by the smooth muscles (Fig. 26a & b).

In the Aroclor 1254-treated animals, the typical tubuloalveolar organization was changed to a great extent (Fig. 26c & d). The lumen of the glands were either collapsed or much reduced in size and was lined with a single layer of cells that had lost their compact arrangement of normal columnar/cuboidal shape due to the disintegration of epithelium. At many places the epithelial cells were detaching and accumulating in the luminal matrix. A dense matrix covered the epithelium. The nuclei were pyknotic. The amount of connective tissue between the acini also increased.

In the prostate glands of Aroclor 1242-treated animals also, the tubuloalveolar epithelium developed abnormalities. The epithelium contained a single layer of cuboidal cells of which a few were hypertrophied and hypochromic and lost their nuclear contents while the others were hyperchromic containing pyknotic nuclei. The nuclei of these cells too were pyknotic. The connective tissue between acini did not increased as much as in Aroclor-1254 treated animals. The epithelial cells were hypertrophied in some places (Fig. 26e & f).

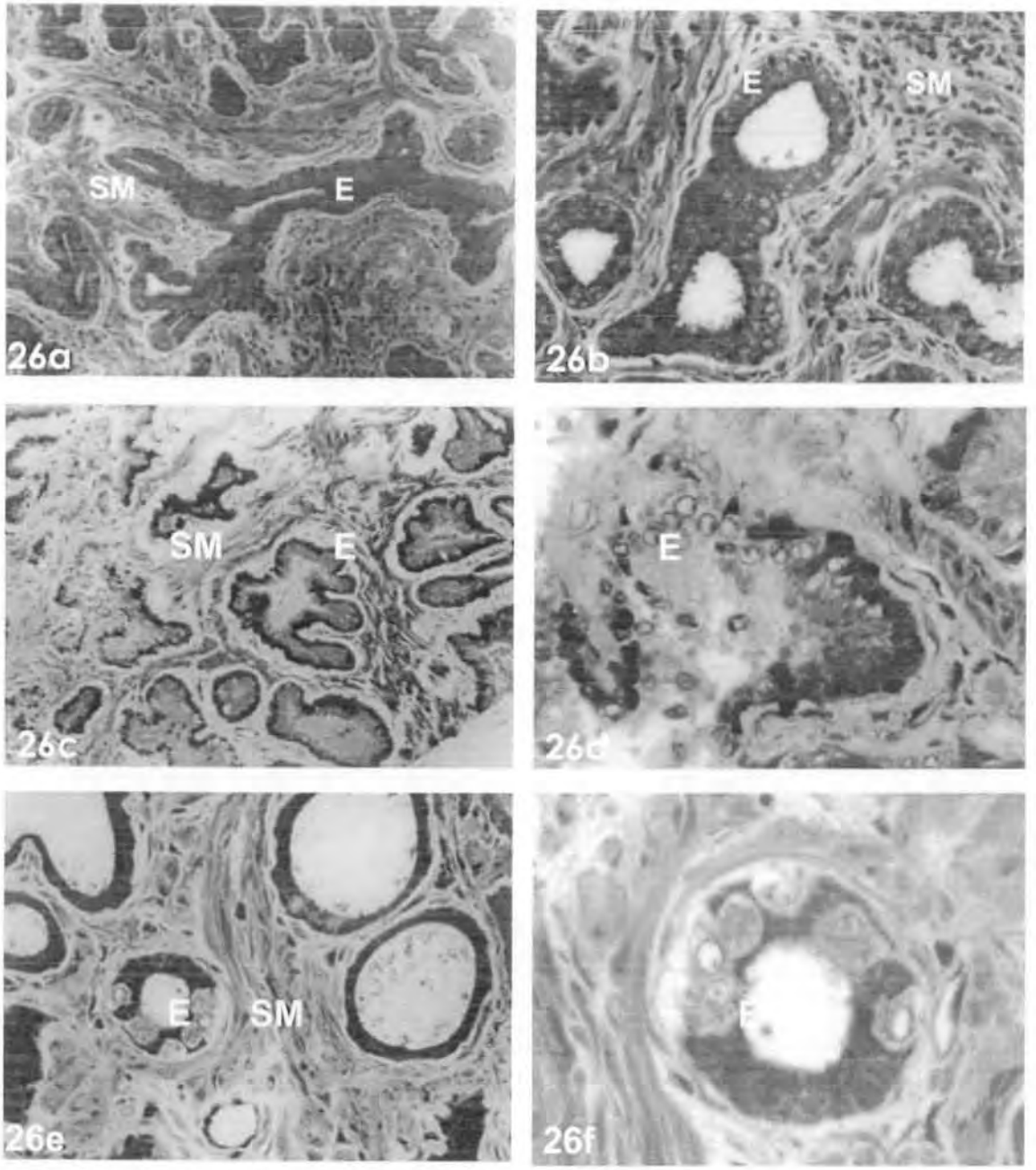


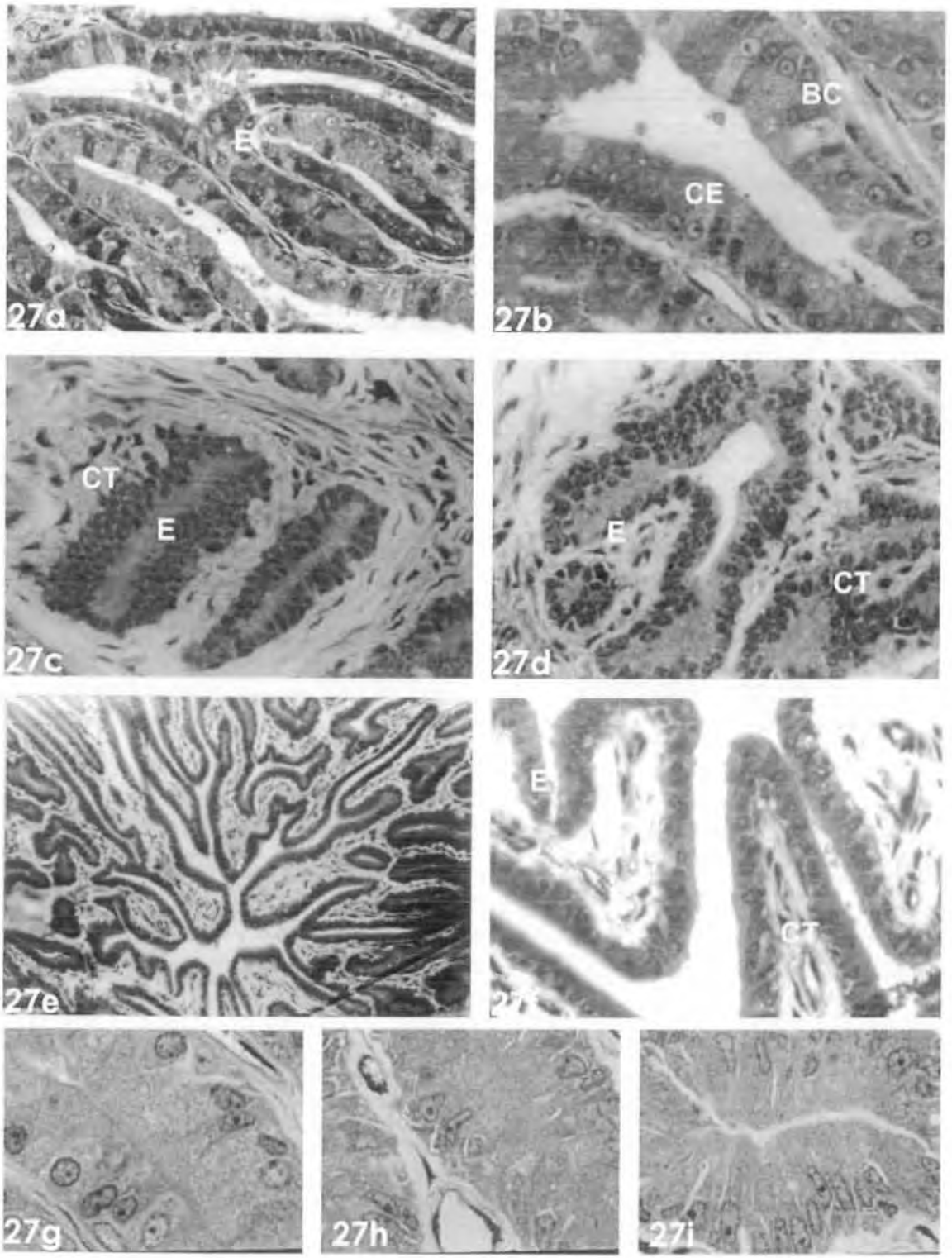
Fig. 26a-f. Photomicrographs of monkey prostate glands. a & b. normal features of stroma and epithelium of vehicle-treated prostate. c & d. Aroclor 1254-treated prostate gland showing epithelial degeneration and tissue necrosis. e & f. Aroclor 1242-treated prostate gland containing hypochromic and hyperchromic cells in the epithelium. A few of them are abnormally large and are squamous not cuboidal. (E; epithelium, SM; smooth muscles)

Seminal Vesicle:

The seminal vesicles of the control monkeys contained highly folded mucosa having a common lumen. The pseudostratified epithelium contained columnar/ cuboidal Principal cells and the small basal cells (Fig. 27a, b & g).

The Seminal vesicles of the Aroclor 1254-treated animals also underwent histological alterations. The mucosal folds showed abnormal organization with an increase in the thickness of epithelium due to stratification at most of the places. Most of the spaces in the gland were occupied by the connective tissue. The nuclei of epithelial cells at many places lost their normal shapes and appeared irregular in shape (Fig. 27c, d & h).

Seminal vesicles of Aroclor 1242-treated animals also underwent histological alterations. Mucosal folds showed irregular organization with stratified epithelium at most places. Columnar epithelial cells became narrow to a great extent and their basally located nuclei also assumed elongated shape. The amount of connective tissue also increased (Fig. 27e, f & i).



Figs. 27a-i. Photomicrographs of monkey seminal vesicles. a & b. Vehicle-treated seminal vesicles exhibiting normal features of the gland. c & d. Aroclor 1254 treated seminal vesicle showing that the amount of connective tissues is increased, lumena have collapsed and the epithelial thickness has increased manifold. e & f. Aroclor 1242-treated seminal vesicle showing irregular organization of mucosal folds. g-i. Cellular features of the epithelium of vehicle-, Aroclor 1254- and Aroclor 1242-treated seminal vesicle sections at 100 magnifications respectively. Note the difference of cell and nuclear properties in all three cases. (BC; basal cell, CE, columnar epithelium, CT; connective tissue, E; epithelium)

DISCUSSION

Adult male rhesus monkeys (*Macaca mulatta*) were administered Aroclor 1254 nd Aroclor 1242 orally at a dose of 200 µg/Kg/day for 6 months in the present study. his dose approximates a dietary level of 5 ppm, which is the concentration of Aroclor 248 reported to cause overt signs of toxicity in rhesus monkeys following a 2-6 month est (Barasoti *et al.*, 1976) and has been known to produce similar clinical symptoms in ynomolgus monkeys, *Macaca fascicularis* (Arnold *et al.*, 1990). Aroclor 1254 and roclor 1242 used in this study are the commercial PCB mixtures of great interest ecause the chromatographic pattern of PCBs found in specimens of human organs nd in various food items is most similar to these mixtures (Arnold *et al.*, 1993).

In the present study, both Aroclor 1254-treated and Aroclor 1242-treated groups n general developed clinical signs of toxicity including swelling of eyelids, edema below ne eyes, hair loss and lesions in the nail-beds. Appearance of dermatological toxicity ymptoms such as follicular epithelial hyperplasia, blockage of the sebaceous ducts, eratinzation of the hair follicles, inflammation of the Meibomian glands, loss of eye ashes, release of eye exudates, and changes in fingernails, toenails and nail-beds, lopecia, acneform lesions, enlargement of tarsal glands and conjunctivitis from PCB eatment in monkeys have also been reported, in previous studies (Allen and Norback, 973; Allen *et al.*, 1974; Barasoti *et al.*, 1976; Ohinishi and Kohno, 1979; Yoshihara *et l.*, 1979; Hori *et al.*, 1982; Truelove *et al.*, 1982; Tryphonas *et al.*, 1984; Kunita *et al.*, 985; Arnold *et al.*, 1990/1993). Such toxicity symptoms have been observed in ionkeys even after administration of as low a dose as 30-50 mg PCB (Yoshimura and)shima, 1971) this shows that only a short exposure to PCBs is required to stimulate ermatological toxic symptoms. Many of these clinical symptoms are similar to those iat appeared in the Yusho and Yu Cheng patients (Kuratsune *et al.*, 1971; Higuchi, 976; Kuratsune and Shaprio, 1984; Lu and Wong, 1984; Urabe and Ahasi, 1984).

The animals under PCB treatment in the present study developed pathological ymptoms during the course of treatment including loss of appetite, diarrhea, lethargy,

ever and vomiting. The frequency of these pathological conditions increased with the assage of treatment period. Appearance of these pathological conditions in both roclor 1254-treated and Aroclor 1242-treated animals may be attributed to the fact that CBs affect most severely the immune system in monkeys (Truelove *et al.*, 1982; ryphonas *et al.*, 1984).

In general, the animals treated with both Aroclor 1254 and Aroclor 1242 exhibited ome neurobehavioral changes in the later part of the treatment period. Such changes ere not exhibited by their vehicle-treated counterparts. These changes included thargy, depression and anger. Neurobehavioral effects may arise from complex nteractions between neuroendocrine and neurophysiological systems. A number of CB congeners have been reported to decrease dopamine levels in the frontal cortex of dults resulting in spatial learning/memory deficits in monkeys (Levin *et al.*, 1988, 1992; chantz *et al.*, 1992; Seegal *et al.*, 1991, 1994; Seegal and Schantz, 1994). urthermore, Aroclor 1254 is known to alter hypothalamic serotonin (5-ydroxytryptamine, 5-HT) content, release of pituitary gonadotropin II (Gn II) *in vitro*, nd inhibits hypothalamic tryptophan hydroxylase (TPH) activity without any effect on nonoamine oxidase (MAO) activity in a fish, Atlantic croaker (*Micropogonias ndulatus*), given at 1 μg/kg bw for 30 days (Khan and Thomas, 2000). Although in the resent study, no specific instrument could be used to evaluate the neurobehavioral ffects of the pollutant, it seems likely that PCBs have exerted negative effects on the eurochemistry of the treated animals.

The magnitude of toxicity was generally greater in the slim as compared to the ulky animals. It is probable that because of their lipophilic nature, PCBs get trapped in dipose tissues with only low amounts reaching the other organs including the skin. his conclusion receives support from a previous study where the male monkeys fed a iet containing 5.0 ppm of PCB gained more weight and attained higher levels of PCB in ne adipose tissue than their female counterparts. The female monkeys developed signs f PCB intoxication within 2 months whereas only minor skin alterations developed in nales even after 1 year inferring that since the males were more bulky than the

emales, they had a greater quantity of adipose tissue in which the ingested PCB could e sequestered (Barasotti *et al.*, 1976). Platonow and Karstad (1973) described similar ifferences in the toxic response of mink to PCB, noting that the male mink were more ulky than the females. The former, at all treatment levels, were less severely affected, urvived longer and produced abundant viable spermatozoa throughout the experiment.

Members of the PCB family are reported to exhibit both estrogenic and ntiestrogenic activities. Uterotropic activity in immature or ovariectomized adult female odents has been accepted to reflect standard estrogenic activity (Hansen, 1998), while n the male animals disruption of androgen production is considered to be an estrogenic esponse (Loomis and Thomas, 2000). Generally, the lower chlorinated hydroxy netabolites (OH-CBs) possess estrogenic activities, whereas the higher OH-CBs have been considered to be antiestrogenic (Hansen, 1998). In the present study, treatment of the monkeys with 200 μg/Kg/day Aroclor 1254 for 6 months failed to decrease estosterone levels during the treatment period. This finding also coincides with the fact hat the Leydig cells which appeared morphologically normal as judged from the large number of lipid droplets, abundance of mitochondria and smooth endoplasmic eticulum; a sign of steroidogenesis. A similar observation has been made for the emale hormones by Arnold *et al.*, (1993) who exposed female rhesus monkeys to ncreasing doses (0, 5, 20, 40, 80 μg/Kg bw) of Aroclor 1254 for 37 months and did not btain significant changes in the serum estrogen and progesterone levels even when erum cholesterol levels declined. In rodents, as high as 25 mg/Kg bw dose of Aroclor 254 for 15 weeks did not affect testosterone (T) levels in the serum of rats (Gray *et al.*, 993) and administration of the PCB congener, 2,2',4,4',5,5'-hexachlorobiphenyls (a onstituent congener of Aroclor 1254), did not influence serum T or the biosynthesis of *in vitro* in mice (Johansson, 1987). This indicates that Aroclor 1254 does not affect eydig cells negatively. Further support to this hypothesis is derived from the study of oomis and Thomas (2000), who in a comparative study assessed estrogenicity of arious environmental contaminants in terms of inhibiting androgen production in the tlantic croaker and could not demonstrate that Aroclor 1254 acts as a xenoestrogen. nterestingly in the same study, a hydroxylated polychlorinated biphenyl (2,2',5'-

richloro-4-biphenylol) and estradiol significantly decreased androgen production by the Leydig cells, and thus exhibited strong estrogenic properties. The present investigation too provides support to the hypothesis that Aroclor 1254 does not mediate its toxic effect through estrogen receptor.

The present study further showed that testosterone levels in Aroclor 1242-treated animals generally declined during the treatment period. The Leydig cells of Aroclor 1242-treated testes exhibited a number of histological and ultrastructural abnormalities. These included the considerable shrinkage of cells and nuclei, deposition of highly electron dense plaques in the cytoplasm, its vacuolation and even loss in some cells. In rodents too Aroclor 1242 has been found to cause decline in testosterone levels. In one study, lactating female rats receiving daily subcutaneous injections of 80 μg or 8 μg of Aroclor 1242 in corn oil, showed significant reduction in serum testosterone levels and lowered LH-stimulated testosterone production per testis and per Leydig cell in the offspring as compared to the corn oil-treated controls (Kim et al., 2001).

Aroclor 1242 has been found to be estrogenic by a number of investigators. Administration of Aroclor 1242 to immature female Sprague Dawley rats significantly increases uterine weights and cell proliferation in the uterus (Krishin and Safe, 1993). Co-administration of Aroclor 1242 and 3,3',4,4' TCB to immature female Sprague Dawley rats with previous experience of increased uterine weights and cell proliferation under the influence of Aroclor 1242 or estradiol results in attenuation of uterine weight, (Krishin and Safe, 1993). Aroclor 1242 is also known to enhance responsiveness of cultured anterior pituitary cells to gonadotropin-releasing hormone in a manner comparable to that observed in estradiol-treated pituitary cultures (Jansen *et al.*, 1993). OH-PCBs such as 4-hydroxy-2',4',6'-trichlorobiphenyl and 4-hydroxy-2',3',4',5'-tetrachlorobiphenyl have been shown, using the production of vitellogenin (an egg yolk protein precursor in oviparous animals as a marker of hepatic ER binding) to have agonist or antagonist interactions with estrogen receptors (ERs) in rainbow trout (Carlson and Williams, 2001). These findings suggest that predicting the estrogenic

effects of a complex mixture of PCBs requires determination of net effects of all estrogenic and antiestrogenic congeners present in the mixture (Battershill, 1994).

PCBs exert their toxic effects on Leydig cells at many levels of steroidogenesis. PCBs directly inhibit both the synthesis of testosterone by the Leydig cell *in vitro* (Freeman and Sangalang, 1977) and the *in vitro* binding of steroid hormones to their intracellular receptors (Lundholm, 1988). Aroclor 1248 (a mixture of tri-, tetra- and penta-chloro congeners) decreases serum androgen levels by interfering with steroidogenesis at conversion of progesterone to testosterone level when administered intraperitoneally (*ip*) at a dose of 10 mg/Kg bw or bilaterally intra-testicular (*it*) at a dose of 25.5 mg/Kg bw after 24 hours injection. This effect is produced due to reduced activity of P450 $_{c17}$. Furthermore, Aroclor 1248 also inhibits serum androgen when it is added *in vitro* for 10-15 minutes only. This clearly indicates that this response is not mediated through Ah receptors, since the interference of Aroclor 1248 on steroidogenesis in testicular tissue cannot be explained by the cytotoxicity of this mixture, which should otherwise affect all steps in the process since Aroclor 1248 did not have an effect on the viability of cells in times during which over 60% inhibition of progesterone-supported androgen production was observed (Andric *et al.*, 2000).

The interference of PCBs with testicular androgenesis is complex and specific. Aroclor 1248 also inhibits the activities of several other enzymes including 3βHSD, NADPH-P450 reductase and 17βHSD. However, it does not decrease the activity of P450 $_{c17}$ activity in the microsomal fractions of the testes of guinea pigs. On the other hand, it inhibits P450 $_{c17}$ activity in the adrenal gland causing decreased production of 11-deoxy cortisol and 11-deoxy cortisone (Goldman and Yawetz, 1992). In interstitial cell preparations of rat, however, a PCB mixture of ortho-isomers and congeners with high chlorine content decreases the activity of P450 $_{c17}$ leading to attenuation of progesterone–supported androgen production (Kovasevic *et al.*, 1995). In addition, Aroclor 1260, in combination with two substitute transformer fluids (the silicone oil-based DC561 and the mineral oil-based ENOL C), markedly decreases serum androgen levels 24 hr after single (*ip*; 10 mg/kg bw) or bilateral (*it*; 25 μg/testis)

dministration. When it is injected or added *in vitro*, the mixture inhibits 3ss-ydroxysteroid dehydrogenase (3ssHSD), stimulates P450 $_{c17}$, and does not affect 7ss-hydroxysteroid dehydrogenase in testicular post-mitochondrial fractions (Andric *et l.*, 2000).

The present study demonstrates that oral administration of polychlorinated iphenyl (PCBs; Aroclor 1254 & Aroclor 1242) at a dose of 200 µg/Kg/day to adult male hesus monkeys causes severe structural alterations in the testes and accessory rgans. The severity of toxicity is evidenced from the fact that the various components f the germinal epithelium (spermatocytes, spermatids and different types of permatogonia) were completely absent in the testicular sections of the PCB-treated nimals. The structural damage caused by the Aroclor 1254 and Aroclor 1242 was ound to be generally of similar nature. However, the severity of damage was greater in he Aroclor 1254-treated testes where most of the components of the germinal pithelium were lost. The spermatogonia of Aroclor 1254-treated testes differed from hose of Aroclor 1242 treated testes in many respects. The spermatogonia of Aroclor 254-treated animals were abnormally larger in size with poor cytoplasmic properties in nost of the sections from all four Aroclor–treated testes. These cells had completely uchromatic, hypertrophied and round nuclei. On the other hand, Aroclor 1242-treated permatogonia exhibited shrinkage of cytoplasm with a lot of vesiculation/vacuolation nd distorted mitochondria. The nuclei too were highly pyknotic at many places.

Sertoli cells also showed a number of structural aberrations in the present study n both treated groups. In general these included shrinkage and crowding of cells and eposition of fat peripherally, presence of poor cytoplasmic machinery and damage to ctoplasmic specializations (ES). The ectoplasmic specializations of the Sertoli cell ave been described as actin-associated adhesion junctions consisting of hexagonally acked filamentous actin bundles sandwiched between the Sertoli cell plasma nembrane and smooth endoplasmic reticulum and have been postulated to stabilize an dhesive membrane domain. It is a unique junctional structure involved in the nteraction between elongating spermatids and the Sertoli cells and is also found on

either side of the junctional complex that is formed between the basal end of the Sertoli cells. This inter-Sertoli cell ES complex which also contains gap and occluding junctions, restricts the passage of macromolecules and forms the basis of the blood-testis barrier that divides the seminiferous epithelium into basal and adluminal compartments. It is hypothesized that the detachment of round spermatids from Sertoli cells may be due to the absence or damage to the specialized ES structure of the Sertoli cells in the seminiferous epithelium (O'Donnell *et al.*, 2000). Furthermore, *in vitro* treatment of cultured Sertoli cells isolated from 19- to 21-day old male rats with hydroxylated PCB congener (3,3',4,4'-tetrachlorobiphenyl) at concentrations $10^{(-8)}$ M causes progressive damage. It kills 45% of the Sertoli cells in culture 24-hours post-treatment. In the remaining cells, observed abnormalities included increased lactate production and disorganization and less intense F-actin staining. However, another congener (2',3',4',5'-tetrachloro-4-biphenylol) given at concentration of $10^{(-7)}$ M does not kill a significant number of Sertoli cells. This shows that different congeners have different cytotoxic effects on cellular systems (Raychoudhury *et al.*, 2000.).

The present study strongly favors the hypothesis that PCBs may cause aspermia in monkeys, decrease sperm motility and quantity of sperms and reduce semen quality in humans. These observations receive strong support from the work of Bush *et al.*, 1986), Nessel and Gallo (1994) and Rozati *et al.*, (2000). Morphological alterations in the reproductive organs of male monkeys in the present study, especially the cessation of spermatogenesis and histological damage to the accessory glands are comparable to reports of destruction pattern of some other xenobiotics in rats and other animals (Boockfor *et al.*, 1997; Khan *et al.*, 1998). However, similar evidence of toxicity is not available for primates except in the mammalian liver that has been widely studied ultarstructurally for possible deleterious effects of PCBs (Kimbrough *et al.*, 1972; Kaszy *et al.*, 1978; Lin *et al.*, 1979; MacLellan *et al.*, 1994a,b,c; Singh *et al.*, 1996, 1997; Peng *et al.*, 1997). It seems likely that in the rhesus monkey, PCBs bring about their toxic effects through Ah receptors. It is however, noteworthy that while Aroclor 1254 does not have an estrogenic effect, Aroclor 1242 elicits an estrogenic response in the rhesus monkey. Battershill (1994) has opined that it is not possible to apply the TEF model to

he evaluation of the effects of PCBs on reproduction since there are insufficient data on ndividual PCB congeners to predict the likely effect of a PCB mixture. However, existing data on cytotoxicities of PCBs favor a number of possible actions.

Apoptotic cell death is an active process, which is a critical feature of the regulated development of multicellular organisms. It has been found that the PCB congener, 2,2', 5,5'-tetrachlorobiphenyl, induces apoptosis in human neuronal cells. The capability of 2,2', 5,5'-tetrachlorobiphenyl to induce apoptosis is associated with the proteolytic cleavage of specific target proteins, such as poly (ADP-ribose) polymerase (PARP) and beta-catenin proteins, suggesting possible involvement of caspases in the process. In general, DNA-damaging agents induce accumulation of the tumor suppressor protein p53, resulting in arrest of cell growth in G1, or apoptosis. However, p53 levels decrease in a time-dependent manner during apoptosis after exposure to 2,2', 5,5'-tetrachlorobiphenyl (Hwang *et al.*, 2001).

It has been reported that skeletal muscle differentiation is specifically impaired by PCBs. Myogenic cell cultures are highly sensitive to PCBs and allow the detection of biological effects of environmental levels of these pollutants. Ultrastructural observations of myogenic cells demonstrate that Aroclor 1254 prevents the accumulation of contractile filaments while inducing hypertrophy of the smooth endoplasmic reticulum and appearance of membrane-filled autophagosomes. Aroclor 1254 inhibits the fusion of L6 myoblasts into multinucleated myotubes and increases creatine kinase (CK) activity dose-dependently, with no effect on cell density which provides evidence for the observations that the offspring of PCB-exposed mothers (both in humans and rodents) are found to have reduced body mass, which is because of the effects of PCBs on differentiation of both a myogenic cell line and primary myogenic cell cultures (Coletti *et al.*, 2001). Furthermore, it has been shown that at least a portion of the PCBs is metabolized through arene oxide intermediates which are capable of producing necrogenic, carcinogenic and mutagenic changes in mammalian tissues (Van Miller & Allen, 1975).

The neuroendocrine system plays a very important role in the control of eproduction and the effects of PCBs on neurochemistry may also add to the overall nagnitude of reproductive toxicity. Evidence for toxic effects of PCBs on ieurochemistry has been provided by a recent report where Aroclor 1254 exposure at a lose 1 μg/day/g bw in the diet for 30 days to Atlantic croaker (*Micropogonias undulatus*) :aused a significant decrease in hypothalamic 5-hydroxytryptamine (5-HT) :oncentrations and inhibition of hypothalamic tryptophan hydroxylase (TPH) (the rate-miting enzyme in 5-HT synthesis) but did not alter the activity of monoamine oxidase (a :atabolic enzyme). Further, PCB treatment caused significant decreases in GnRH :ontent in the preoptic-anterior hypothalamic area of this fish. Significant decreases in ituitary GnRH receptor concentrations and the LH response to the GnRH analogue GnRHa) have also been observed in the PCB-exposed fish, possibly as a :onsequence of a decline in GnRH release. The possible association between impaired erotonergic and neuroendocrine functions in the Atlantic croaker after PCB treatment vas explored using serotonergic drugs. Treatment with p-chlorophenylalanine (an rreversible TPH inhibitor) mimicked the effects of PCB on the GnRH system and the LH esponse to GnRHa. Bypassing the TPH-dependent hydroxylation step with dministration of 5-hydroxytryptophan restored 5-HT to the control levels and prevented he deleterious effects of PCB on the neuroendocrine parameters. Moreover, slow elease from GnRH implants prevented the PCB-induced decline in GnRH receptors nd restored the LH response to GnRHa, suggesting that GnRH therapy can reverse CB-induced disruption of LH secretion. These results demonstrate that TPH is one of he targets of PCB neurotoxicity and a decrease in 5-HT availability in PCB-exposed roaker results in disruption of the stimulatory 5-HT/GnRH pathway controlling LH ecretion (Khan and Thomas, 2001).

In the present study, accessory organs of both treated groups (Aroclor 1254 and roclor 1242) also developed abnormal features in the epithelium, an increase in mount of connective tissue and collapse of luminal areas after PCB exposure. Gray et l., (1993) have reported that in rats, Aroclor 1254 at 10 and 25 mg/kg/day doses, auses severe physiological alterations and significant reductions in the number of

sperm stored in the cauda epididymis. This treatment decreases accessory gland weights in the absence of an effect on serum testosterone. In another study, 6-CB (PCB congener) failed to reduce serum testosterone levels or its synthesis *in vitro* in mice without affecting the accessory gland weights, demonstrating that the reductions in seminal vesicle weight are not a consequence of reduced serum levels of testosterone (Johansson, 1987). However, Orberg and Lundberg (1974) have opined that the effects of PCBs on the size of accessory sex gland and sperm production are a result of increased testosterone turnover. This shows that a number of alternative mechanisms exist for the effects of PCBs on male reproductive system. For example, some extra gonadal hormones play a role in the maintenance of functions of the seminal vesicle (Dadoune, 1985). An altered thyroid state influences androgen-regulated glycolytic enzymes in the seminal vesicles, thereby affecting the secretory activities of this organ It is also possible that PCBs inhibit critical enzymes such as (5-α-reductase) in seminal vesicles. Since Aroclor 1254 contains tetrachlorobiphenyls, which repress the hepatic enzyme 5-α-reductase (Dieringer et al., 1979), it is possible that Aroclor 1254 inhibits the formation of the active androgen, 5-α-DHT, by inhibiting 5-α-reductase in the accessory sex glands. Seminal vesicle growth also requires growth hormone and insulin for its normal functioning, while anti-androgens, inhibitors of prostaglandin synthesis, inhibitor of prolactin synthesis and corticosterone inhibit the growth of seminal vesicles. PCBs have been shown to alter serum corticosterone (Bryne *et al.*, 1988). Elevated corticosterone levels are associated with chronic stress and reduce the size of accessory sex glands in rodents (Brain, 1972; Christian and Davis, 1971). Therefore, reproductive alterations have generally been attributed to hormonal disturbances caused by the induction of hepatic enzyme systems (Orberg and Lundberg, 1974). PCBs are potent inducers of many species of cytochrome P 450, which metabolize steroid hormones. For example, when castrated, testosterone-treated mice were treated with PCBs, hepatic cytochrome P 450 content and liver weight were increased, along with a decrease in the dry weight of the seminal vesicles (Dierniger *et al.*, 1979).

The management of PCBs has attained much attention. A number of mechanisms have been discovered for the detoxication of PCBs in the environment

ncluding the development of cellular biosensors (biological monitor that recognizes a hemical or physical change and produces a measurable signal in response to the nvironmental change) (Daunert *et al.*, 2001), or by the use of biphenyl degrader nicrobes such as Rhodococcus sp. strain RHA1 which efficiently degrades PCB nixtures into intermediate metabolites such as di- and trichlorobenzoic acids which are urther bioaugmented by certain other species of bacteria destructing metabolites ompletely (Seto *et al.*, 1995; Quenson and Tiedje, 1998). Photodegradation is still nother way of PCB decomposition by using sensitizers in order to enhance legradation process as most of PCB congeners do not strongly absorb wavelengths bove 300 nm (Lin *et al.*, 1995). The rate and degree of photodegradation can be nhanced by the use of lower wavelength ultraviolet light that leads to hydroxylation of PCB congeners increasing solubility and accessibility to photocatalytic reactions Chiarenzelli *et al.*, 1995).

Therefore, the management of PCBs in the environment needs effective biomonitoring ystems and use of best available science. A balanced and comprehensive assessment f the data is necessary to determine the geographic extent of exposure and eproductive effects associated with environmental pollution. However, initial efforts to document reproductive injury should focus on specific ecosystems in which detrimental effects have been observed. Model systems (including experimental mesocosms or field ecosystems) should be identified or designed that can adequately test multigenerational reproductive effects. Mechanistic data from supportive laboratory studies on eproductive toxicity, quantitative structure-activity relationships, and bioaccumulation an be used to predict effects of related pollutants and to determine risk. Such nformation is essential to prevent future injury to humans and wildlife and to prioritize he numerous remediation decisions facing our society.

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Environmental pollutant, Aroclor 1254 (PCB) Treatment Disrupts Spermatogenesis in the Adult Rhesus Monkey (*Macaca mulatta*)

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Summary:

Adult male rhesus monkeys (*Macaca mulatta*) were given oral dose of 200 μg/kg/day/animal of Aroclor 1254 for six months to examine its effect on spermatogenesis and accessory gland cytology. Serum testosterone concentration in weekly samples of blood was determined by radioimmunoassay. Body weights and testicular volume of the treated animals decreased significantly. PCB treatment did not affect testosterone secretion, and Leydig cells appeared normal. Aroclor 1254 administration decreased the number and increased the size of spermatogonia. Shrunken Sertoli cells containing fat droplets were widespread in the cords. In the epididymides, the lumen was either reduced in size and lacked spermatozoa or was completely obliterated owing to increase in epithelial thickness and stratification. In the prostate glands, Aroclor 1254 markedly changed the typical tubuloalveolar organization. The lumen was either collapsed or much reduced in size and the epithelium contained pyknotic nuclei. In the seminal vesicles, the mucosal folds became stratified and irregular in organization. It is concluded that Aroclor 1254 has adverse effects on the gonads and accessory glands without altering testosterone secretion in the adult male rhesus monkeys.

Introduction:

Polychlorinated biphenyls (PCBs) are the commercial mixtures of

congeners. As highly persistent toxicants, these chemicals accumulate in trophic hierarchy of global ecosystem (Safe, 1993; Wester *et al.*, 1993). Several recent reviews have recapitulated PCB toxicity studies made during the last three decades (Battershill, 1994; Hansen, 1998; Tilson, 1998 and Fischer *et al.*, 1998).

Aroclor 1254 is a mixture that includes 3,3', 4,4'-tetra-, 3,3', 4,4', 5 penta- and 3,3', 4,4', 5,5'-hexachlorobiphenyl congeners (Mes *et al.*, 1995). Its concentration increases in the monkey blood and tissues in a dose-dependent manner (Tryphonas *et al.*, 1986). In rhesus monkey, this pollutant significantly decreases conception rate and increases fetal mortality (Arnold *et al.*, 1995) besides causing developmental problems such as abortions and immunologically impaired dam births in impregnated monkeys (Barasoti *et al.*, 1976; Truelove *et al.*, 1982). This study describes toxic effects of Aroclor 1254 on male reproduction in rhesus monkey (*Macaca mulatta*).

Materials and methods

Eight adult (5-7 year old) male rhesus monkeys (*Macaca mulatta*) were purchased from local suppliers. The animals were housed in individual stainless steel cages under standard colony conditions (temp; 24±2°C, 12:12 light/dark cycle). The animals were quarantined for two months. They were given oral treatment of either Aroclor 1254 (Electrical Grade; Lot KB05-612; Monsanto Company, St Louis, MO, USA) mixed with vehicle (corn oil and glycerol) at a dose of 200 µg/kg/day/animal (n=4) or vehicle only (n=4) for a period of six months in self-ingesting gelatin capsules. Body weight and testicular size were noted and blood samples (3 ml) were collected by venipuncture from Ketamine sedated animals on weekly basis. Following sacrifice using humane care (euthanasia; sodium penta barbital), testis and accessory glands (epididymides, seminal vesicles and prostrates) were removed, cleaned and weighed.

The tissues were fixed in 2% Glutaraldehyde, post-fixed in 1% Osmium tetroxide (Sigma Chemical Co., St Louis, MO, USA) and embedded in Epoxy resin media (LX-112; LADD Research Industries Inc., USA) using standard procedures. Semithin sections were made with glass knives on LKB ultratome, stained with 1% toluidine blue and studied under Nikon Optiphot light microscope. Testosterone was determined by radioimmunoassay and the data were analyzed by Student t test.

Results

Body weights and testicular diameter of Aroclor 1254-treated animals decreased significantly ($P<0.05$) during the treatment period.

Testosterone levels remained unchanged in both Aroclor 1254 and vehicle treated groups. There was no significant difference in the

testosterone levels of treated animals when comparisons were made between the earlier half and later half of the treatment periods.

General Histology

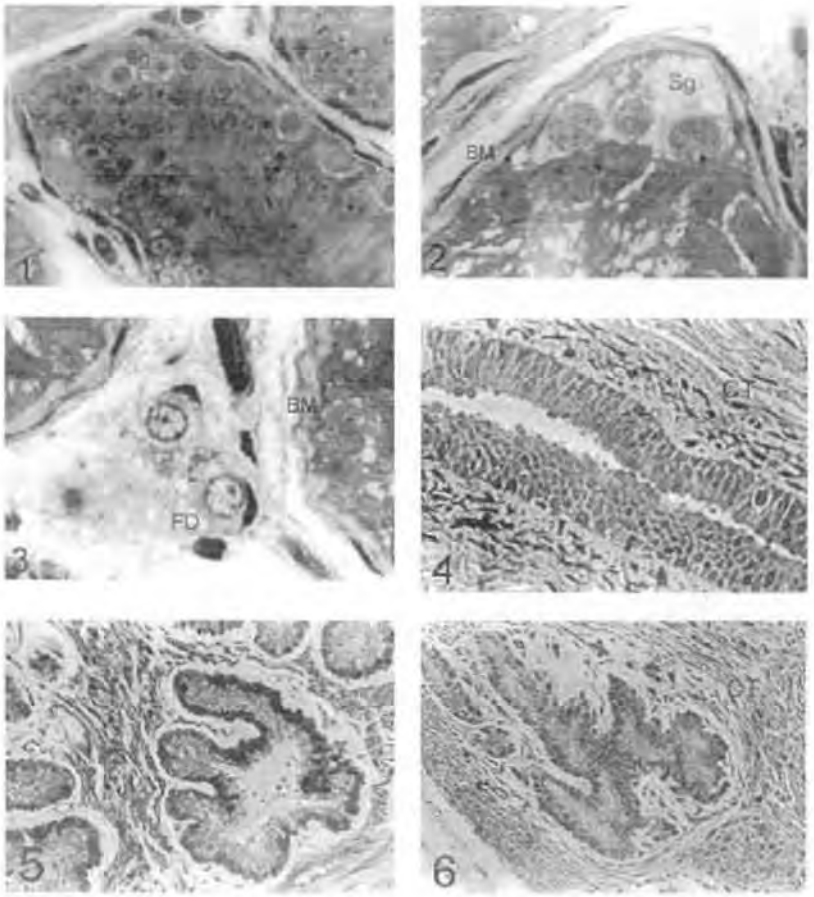
Testes

Aroclor 1254 caused cessation of spermatogenetic activity in the testes (Fig.1). In the absence of spermatogenetic stages, the Sertoli cells became more pronounced. The tunica propria became wavy and indented. A conspicuous gap separated the unusually thickened basement membrane and the peritubular Myeoid cell layer (Fig. 2). The Myeoid cells too appeared necrotic and contained deeply stained pyknotic nuclei. The basement membrane of many tubules appeared thickened. Seminiferous cords lacked the typical germinal epithelial organization in Aroclor 1254 treated animals where the number of spermatogonia was far less than in the vehicle treated animals. Among these, a few were abnormally large in size (Fig. 2) and in many instances contained empty spaces due to shrinkage of cytoplasm. The Ap type spermatogonia, resting on the basement membrane were more conspicuous than the sparsely stained B spermatogonia. The spermatogonia contained round or oval-shaped nuclei.

The Sertoli cells appeared shrunken, containing a lot of fat droplets basally. In most of the sections, luminal areas of the cords were occupied by the cytoplasmic extensions of Sertoli cells (Fig. 1). At some places these cells were unusually crowded. The typical irregular shaped nuclei of these cells were completely euchromatic with prominent nucleoli. Leydig cells were unaffected by Aroclor 1254 treatment. These cells were scattered randomly in the interstitium. These cells had round nuclei with variable heterochromatic portions. Their cytoplasm contained numerous lipid vacuoles (Fig. 3).

Accessory organs

In the epididymides, the Aroclor 1254 caused increase in the amount of connective tissue, and epithelial thickness and reduction in size of luminal space that lacked spermatozoa. The epithelial cells became necrotic and lost stereocilia (Fig. 4). This was in sharp contrast to the control group where pseudostratified columnar epithelial cells having stereocilia were pronounced. In the prostate glands of the Aroclor 1254-treated animals, the typical tubuloalveolar organization was completed changed (Fig. 5). The gland's lumina were either collapsed or reduced in size. The single-layered alveolar epithelium contained cells that had lost their normal cuboidal/squamous shape due to shrinkage. The nuclei of these cells became pyknotic. The connective tissue between t he acini increased in



Figs. 1-6: Photomicrographs of Aroclor1254-treated monkey testes and accessory organs. 1) Seminiferous cord showing an overview of damage caused by Aroclor 1254. Only Sertoli cells and a few Spermatogonia are visible. 2) Abnormally large sized spermatogonia. 3) Leydig cell with fat vacuolation in the cytoplasm. 4) Epididymis. 5) Prostate and 6) Seminal vesicles of Aroclor 1254 treated monkeys respectively (BM: basement membrane; CT: connective tissue; FD fat droplets; L: Leydig Cell, M: Myeoid Cell; S: Sertoli cell; Sg: spermatogonia)

amount. In the seminal vesicles, Aroclor 1254 induced irregular organization of mucosal folds, hyperplasia of epithelial cells, loss of columnar character and increase in interstitial connective tissue (Fig. 6).

Discussion and conclusion

The present study reveals that chronic treatment of the adult rhesus monkey with Aroclor 1254 at a dose of 200 µg/Kg/day causes severe structural alterations in gonads and accessory organs. The effect on the somatic compartment of the testis is less pronounced as compared to the germinal compartment where the damage is quite drastic. This strongly supports the view that PCBs cause aspermia in monkeys (Nessel and Gallo, 1992) and may be the causative agents for decreased sperm motility in humans (Bush *et al.*, 1986).

In the present study, the mechanism of toxicity does not seem to be estrogenecity since pollutant failed to curtail testosterone levels, a characteristic feature of effects of estrogenic xenobiotics on male reproduction. The normal histology of Leydig cells also coincided with lack of effect on serum testosterone concentration in the Aroclor 1254 treated monkeys. The testosterone levels remained statistically similar during the first half and second half of the treatment period. Gray *et al* (1993) have shown that treatment of rats with Aroclor 1254 at a dose of 25-mg/Kg body weight for 15 weeks does not affect serum testosterone levels. These observations collectively suggest that Aroclor 1254 causes damage to the testes directly and its action is not mediated through down regulation of testosterone.

PCBs are also considered as xenoestrogens along with other environmental contaminants because of their capacity to mimic natural estrogens. However, all isomers or mixtures of PCBs are not estrogenic. Loomis and Thomas (2000) have shown in a study on Atlantic croaker that while Aroclor 1254 lacks estrogenecity, hydroxylated polychlorinated biphenyl (2,2', 5'-trichloro-4-biphenylol) significantly decreases androgen production by Leydig cells, exhibiting strong estrogenic properties.

Further diversity of PCB toxicity is evident from the study of Andric *et al* (2000) who have demonstrated that Aroclor 1248 (a mixture of tri-, tetra- and penta-chloro congeners) decrease serum androgen levels in rats by inhibiting steroidogenesis when administered intraperitoneally at a dose of 10-mg/Kg body weight or through bilateral intra-testicular route at a dose of 25.5-mg/Kg body weight for 24 hours. It has been suggested that Aroclor 1248 decreases the conversion of progesterone to testosterone in post-mitochondrial fraction that may be due to reduced activity of P450 $_{c17}$ (Andric *et al.*, 2000). However, Aroclor 1248 has not been shown to

decrease the activity of $P450_{17}$ in the microsomal fractions of the guinea pig testis. On the other hand, Aroclor 1254 inhibit $P450_{17}$ activity in the adrenal gland causing decreased production of 11-deoxy cortisol and 11-deoxy cortisone (Goldman, 1992). In the interstitial cell preparations of rats, however, PCB mixture of ortho-isomers and congeners with high chlorine content decreases the activity of $P450_{17}$ leading to attenuation of progesterone-supported androgen production (Kovasevic *et al.*, 1995). Such species-dependant and congener-specific differences demand further studies on individual congeners to find the exact mechanism of PCB toxicity.

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