Chapter - 1

INTRODUCTION
1. Introduction

Environmental endocrine disruption-

A special report on environmental endocrine disruption provides an overview of the current state of the science for endocrine disruption, in terms of human health, it focuses on general exposure to chemicals through environment, rather than occupational or pharmaceutical (drug) exposure. There is no single definitive source of information on the toxic effects of pesticides and common industrial chemicals. In fact, according to the EPA, reliable toxicity data exist for only 43% of the 75,000 chemicals currently in common use today. In addition, less than 7 percent of all chemicals in high volume commerce can be considered thoroughly studied, according to the EPA. Fortunately, pesticides and toxic chemicals routinely found in food are among the most well studied pollutants.

Endocrine disrupting chemicals are substances that can cause adverse effects by interfering in some way with the body’s hormones or chemical messengers. These substances are therefore called hormone disrupters or endocrine disrupters, as it is the endocrine glands that secrete the hormones. There is particular concern about endocrine disrupting pesticides that are lipophilic (fat loving), resistant to metabolism, and able to bioconcentrate up the food chain. This is because these substances become stored in body fats and can be transferred to the developing offspring via the placenta or via the egg.
Predator animals (and humans) feeding at the top of the food chain are at increased risk, particularly mammals because during breast feeding contaminants are again mobilised and transferred to the new born infant. The endocrine (hormone) system is the body’s messenger system, linking different organs and organ systems via chemical signals that tell the body everything from when it is time to grow reproductive organs, to when metabolism should be increased.

Hormones effect the functioning or development of many organ systems via a very delicate collection of feedback loops. When the endocrine system is functioning correctly, glands located throughout the body synthesize hormones and secrete them into the bloodstream. Receptors in the cells of various organs and tissues respond to these chemical messages to regulate sexual development, reproduction, metabolism, the brain and central nervous system, and other bodily functions. Tiny amounts of hormones induce powerful responses (like sexual development); just a few parts per trillion difference in the womb can have huge effects on the developing fetus. If any step in this complex chain is altered in the tiniest way, the ultimate response could be drastically different than intended.

Some chemicals can disrupt the transmission of these messages by blocking the hormone receptors in cells, while others inhibit the ability of different glands to create the hormones in the first place. Other chemicals affect the way that these hormones are stored, transported, and eventually destroyed. Endocrine disrupters commonly found in food include DDT and its metabolite DDE, PCB’s, and DEHP, the insecticide endosulfan and its metabolites, the
fungicides vinclozolin, iprodione, and ethylene thiourea, and dioxin.

Malathion is also one of the most widely used organophosphate insecticide in the United States and throughout the world. It is used to control pests of agricultural crops, ornamentals, greenhouses, livestock, stored grain, forests, buildings, households, and gardens. Industrial, commercial, and government applications constitute most of the annual U.S. usage. These uses include schools, hospitals, warehouses, eating establishments, food processing plants, and wide scale pest control or eradication programs. Contributing to its popularity is malathion’s relatively low acute mammalian toxicity. But like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label would lead one to believe. Shown to be mutagenic, a possible carcinogen, implicated in vision loss, causing myriad negative health effects in human and animal studies, damaging to nontarget organisms, and containing highly toxic impurities, malathion has a legacy of serious problems. Malathion has also been associated with birth defects in domestic and laboratory animals. In rabbits, malathion crosses the placenta and acts on the central nervous system.

We use malathion to study its effects on adrenal and gonads of wistar albino rats because it is an organophosphate insecticide, one of the class of pesticide that are highly toxic to vertebrates.
Adrenal steroidogenesis-

The importance of the adrenal cortex relative to medulla was a matter of
debate well into the 20th century the mechanism by which adreno cortical
function is regulated have been elucidated in 20th century. The work of Philip
E. Smith revealed the existence of a functional pituitary – adrenal axis, and D.J.
Ingle and Edward C. Kendall showed in 1937 that an adrenocortical extracts
inhibited the adrenocorticotropic effect of the pituitary, establishing the
presence of homoeostatic negative feedback mechanism characteristic of most
endocrine regulatory systems.

All human steroid hormones are derived from cholesterol. The cells of
steroidogenic tissues can synthesize cholesterol de novo from acetate, mobilize
intracellular cholesterol ester pools, or important lipoprotein cholesterol from
plasma. About 80% of the cholesterol is usually provided by circulating plasma
lipoprotein. Adrenal tissue in vitro utilizes low –density lipoprotein (LDL)
cholesterol via a specific receptor–mediated pathway. Such receptors are present
in mouse adrenal cortical tumor cells and normal adrenal tissue from a variety
of species. Specific cell surface receptors for LDL, localized to structures
called coated pits, bind circulating LDL and internalize it by receptor mediated
endocytosis. The coated pits invaginates to from a coated vesicle containing
receptor–bound LDL. The coated vesicles fuse with lysosomes, were cholesteryl
esters are hydrolized to liberate free cholesterol for use as steroidogenic
substrate.
Figure 4.6. Pathways of adrenal steroid biosynthesis
Adrenal glands of animals other than rat and mouse do not have specific binding sites for high-density lipoprotein (HDL), and most species apparently do not use HDL cholesterol for adrenal steroid biosynthesis.

The adrenal cortex can also synthesize cholesterol de novo from acetyl coenzyme A. Under normal conditions about 20% of steroidogenic capacity depends on intracellular cholesterol biosynthesis. In disorders that impair delivery of exogenous cholesterol, basal adrenal steroidogenesis is normal. Abetalipoproteinemia is hereditary deficiency of apolipoprotein B production in which LDL is absent from plasma. Patients with this disorder have normal basal adrenal steroid hormone production. Patients with familial hypercholesterolemia have defective LDL receptors but have normal steroidogenesis, possibly because elevated plasma LDL levels promote LDL cholesterol uptake by nonspecific pathways. However, even though basal steroidogenesis is unimpaired by defects in extracellular cholesterol delivery, increased steroidogenesis stimulated by prolonged ACTH administration can not be sustained by de novo synthesis of cholesterol alone.

The amount of free intracellular cholesterol available for adrenal steroidogenesis is metabolically regulated, and negative feedback is exerted via the LDL pathway to control the amount of free intracellular cholesterol in adrenocortical cells. LDL uptake suppresses cellular cholesterol synthesis by reducing the activity by hydroxymethylglutaryl-coenzyme A (HMG CoA)
reductase, the rate-limiting enzyme in cholesterol biosynthesis. Esterification of imported cholesterol is stimulated, and cell-surface LDL receptors number is downregulated as a consequence of LDL cholesterol uptake by receptors-mediated endocytosis. ACTH increases the number of LDL receptors on the cell surface, the activity of the cholesterol esterase that liberates free cholesterol from cholesteryl ester delivered by LDL or stored in lipid droplets, and, as consequence, the amount of free intracellular cholesterol. ACTH does not stimulate HMG-CoA reductase activity or alter the ability of LDL to suppress it.

Role of adrenal at different stages of life

Role of adrenal in fetus

A morphologically and functionally distinct fetal zone exists in the human fetal adrenal gland until birth, after which it rapidly involutes. Under ACTH stimulation, the fetal zone imports LDL cholesterol and synthesizes cholesterol de novo to produce mainly pregnenolone sulfate and DHEAS, beginning at about 25 wk of gestation. Predominant secretion of D-steroids such as DHEAS apparently is due to low activity of the 3b-hydroxysteroid dehydrogenase/D-isomerase required to convert 17a-hydroxypregnenolone to 17a-hydroxyprogesterone. DHEAS is converted to 16a-hydroxy-DHEAS in peripheral fetal tissues and serves as substrate for placental estrogen synthesis. Estrogen, in turn, inhibits 3b-hydroxysteroid dehydrogenase activity in the fetal
adrenal, thus perpetuating production of its DHEAS precursor.

**Role of adrenal in placental estrogen biosynthesis**-

The human is one of the few mammals in whom estrogen are produced in large quantities during pregnancy. The adrenals of the human fetus at term are as large as those of adults, weighing 10g or more. Morphologically, however, the fetal adrenal differs from that of the adult. The human fetal adrenal is composed principally of an inner fetal zone that accounts for 85% of the volume of the gland. The outer zone, i.e., the neocortex which ultimately develops into the adult adrenal cortex, makes up 15% or less of the total volume. In addition to its size, the human fetal adrenal has a remarkable capacity for steroidogenesis. Near term, the fetal adrenals secret 100 to 200mg steroid per day. The principle secretory product are dehydroepiandrosterone sulfate and pregnenolone sulfate.

In addition to the role of the fetal adrenal cortex in providing precursors for placental estrogen formation, its secretions may participate in biochemical events that lead to the initiation of parturition and to fetal lung maturation. Therefore the control of steroidogenesis by the human fetal adrenal is an issue of considerable importance in the endocrinology of human pregnancy. The fetal adrenal appears to be responsive to more than one trophic stimulus. First, ACTH levels in human fetal blood decline as gestation advances; paradoxically, the rate of growth of the adrenals increases at a time when ACTH levels are falling. Second, the fetal adrenals secret different steroids from those of adult adrenals.
For these reasons a trophic role has been proposed for peptides such as growth hormones, hCG, prolactin, hPL, and a-melanocyte-stimulating hormone. There is little convincing evidence, however, that any of these proteins hormones serve an important role in stimulating growth or steroidogenesis directly in the fetal adrenal cortex. It is likely that a growth factor stimulates growth of the adrenal without directly enhancing the synthesis of steroidogenic enzyme. The increase in the rate of steroidogenesis during gestation may be caused, at least in part, by the increase in the size of fetal zone of the adrenal.

Attempts have also been made to define the precursors of the steroid hormones synthesized by the fetal adrenal. Some investigators have suggested that circulating progesterone and pregnenolone of placental origine could serve as precursors for the fetal adrenal steroidogenesis. On the basis of the level of pregnenolone (sulfate) in umbilical venous blood, however, it can be computed that this source of steroid precursor accounts for no more than 1% of the dehydroepiandrosterone sulfate secreted by the fetal adrenal. Conceivably a portion of fetal adrenal cortisol is formed by the utilization of progesterone produced within the placenta. However, suppression of fetal ACTH secretion by dexamethasone therapy in pregnant woman leads to a striking decrease in fetal plasma cortisol levels. Moreover, the fetal adrenal gland possesses the capacity for de novo synthesis of cortisol and thus likely does not require placental steroids as precursors for steroid biosynthesis in a quantitative sense.
The principal precursor for fetal adrenal steroid biosynthesis is probably cholesterol. There are two possibilities for the source of cholesterol could be formed in situ in the fetal adrenal by de novo synthesis from two-carbon precursors. Second, cholesterol could be assimilated from plasma lipoproteins.

The LDL cholesterol present in the entire plasma volume of the human fetus near term is only 30 mg. of cholesterol. Thus if LDL were the principal source of cholesterol for fetal adrenal steroidogenesis, its turn over in fetal plasma must be rapid compared with that in the adult, and indeed, lepoprotein cholesterol is the form of cholesterol preferentially utilized for steroidogenesis in human fetal adrenal tissue fragment maintained in organ culture in the presence of ACTH. It thus appears possible that the rate of fetal adrenal steroidogenesis may be regulated in part by the concentrations of LDL in the fetal plasma and hence by the rate of synthesis of lepoproteins in the fetus.

It appears that no more than 20% of fetal cholesterol can be derived from the maternal circulation. As LDL is ultimately derived from very low density lepoprotein (VLDL) after the hydrolysis of the triacylglycerol portion of VLDL by lepoprotein lipase. It may be that the fetal lung is important in LDL formation. This obtains because there is little adipose tissue in the human fetus before the 36th wk of gestation. Lipoprotein lipase activity is present in fetal rat lung tissue and prolactin is known to stimulate lipoprotein lipase in other tissues. Thus, prolactin may act to facilitate adrenal steroidogenesis through stimulation of
the conversion of VLDL to LDL in the fetal tissues. In keeping with this view, fetal plasma prolactin levels increase in a manner parallel to the rate of increase in the size of the fetal adrenal cortex. Thus prolactin may be an indirect trophic agent for the fetal adrenal, even though the hormone does not seem to stimulate fetal adrenal steroidogenesis directly.

Role of adrenal at puberty-

Adrenarche is the onset of menstruation and other physiologic changes of puberty induced by hyperactivity of adrenal cortex. The concentration of the weak adrenal androgen dehydroepiandrosterone (DHEA) increases after age 6, several years before the increased secretion of estrogen, gonadotrophins, and prolactin in pubertal girls (Hopper and Yen, 1975, Ojeda et al, 1980, Cutler and Loriaux, 1980 Reiter and Grumbach, 1982). DHEA appears largely as a sulfato, DHEA-S, in human blood. Another androgen D-4-androstenodion, increase at slightly later ages. Accelerated growth and appearance of pubic and axillary hair are associated with increased blood concentration of adrenal steroids. The hormonal mechanism underlying adrenarche are not known.

But seem to be independent of mechanism that influence gonadal development (Cutler and Loriaus, 1980, Reiter and Grumbach, 1982). Children with gonadal dysgenesis can have normal adrenarche (Boyar et al, 1973).

Adrenal insufficiency in human may delay puberty (Boyar et al 1973)
but this association is not found in all cases (Reiter and Grumbach, 1982), adrenalectomy of rats delays puberty, whereas corticoid replacement restores the normal onset (Ramley, 1978). There is general agreement that adrenal steroids are not obligatory for maturation of hypothalamic controls over the gonad in either sex.

**Adrenal Androgen And Puberty**

The earlier onset of adrenarche than gonadarche and the contribution of adrenal androgens to the growth of pubic and axillary hair have led some to suggest that in normal children adrenal androgens are an important factor in the onset of puberty on the maturation of the hypothalamic-pituitary-gonadal complex. Although true precocious puberty may occur in circumstances in which the prepubertal child has previously been exposed excessive levels of androgens from an endogenous or an exogenous source there is little evidence that adrenal androgens play an important quantitative or rate limiting role in the onset of puberty in normal children.

Most patients with premature adrenarche, who secrete excessive amount of adrenal androgens for their age, enter puberty and experience menarche within the normal age range. Moreover prepubertal children who have congenital or acquired chronic adrenal insufficiency (Addison disease) and consequently have deficient or absent adrenal androgen secretion usually have anormal onset of progression through puberty when given appropriate glucocorticoid and
mineralocorticoid replacement therapy. Thus early activation of adrenal androgen secretion does not commonly lead to sexual precocity, nor is deficient or absent adrenal androgen output usually associated with delayed puberty. Furthermore, growth studies in children with chronic adrenal insufficiency isolated gonadotropin deficiency, hypergonadotropic hypogonadism and androgen resistance (testicular feminisation) suggest that in girls and boys adrenal androgens are not essential for the adolescent growth spurt, whereas gonadal steroids secreted by the testes and ovary are and act in concert with GH. A transient increase in height velocity that occurs in middle childhood and lasts about to years has been attributed by some to adrenarche. However the middle childhood spurt is related to the cyclic pattern of prepubertal growth and to genetic regulation of growth rather than an increase in either adrenal androgen or GH secretion.

Nature and regulation of adrenal androgens-

The major androgens secreted by the adrenal cortex are DHEA, DHEAS, and androstenedione. By extraglandular metabolism, the adrenal androgens contribute to physiologically active testosterone and estradiol. In normal adult women, only androstenedione is an important precursor; DHEA and DHEAS contribute little to plasma testosterone and estradiol. However, scant information is available on the metabolism and kinetics of DHEA and DHEAS in prepubertal children. Androstenedione is the major androgen secreted by the ovary during and after puberty. It is more readily converted to potent androgens than DHEA
or DHEAS. However, DHEA and DHEAS are useful biochemical markers of adrenal androgens secretion and the onset of adrenarche. Cross sectional and longitudinal studies have demonstrated a progressive increase in the plasma concentration DHEA and DHEAS in boys and girls by the age of 7 or 8 (6 to 8 y skeletal age) that continues to age 13 to 15. During this 8 – y period, a 20-fold increase in the concentration of DHEAS is accompanied by increased excretion of urinary 17-ketosteroids, especially 11-deoxy C19-steroids. These increase serves as a mark of the onset of adrenarche and begins approximately 2 y before the increase in gonadotropin and gonadal steroid secretion. The increase is not associated with increased sensitivity of the pituitary gonadotropes to LHRH or with sleep-associated LH secretion and occurs at an age when the hypothalamic-pituitary-gonadal complex is functioning at low level.

Changes in human adrenal microsomal enzyme activity during adrenarche are consistent with the alterations in adrenal androgen secretion. The increase in circulating adrenal androgen levels at adrenarche is associated with arise in adrenal 17,20-desmolase and 17-dextroxylase activities whereas 3b-hydroxysteroid dehydrogenase activity does not change significantly. These alterations in adrenal enzyme activity appear to be responsible for the increase in adrenal androgen secretion at adrenarche.

There are several hypotheses about the control of adrenal androgen secretion.
Cortisol and adrenal androgen secretions very independently with age, during normal as well as premature adrenarche, and in Cushing disease, starvation, malnutrition, anorexia nervosa, and chronic disease.

Unlike cortisol secretion, the secretion of DHEA and DHEAS in response to ACTH administration varies with age.

Dissociation of adrenarche and gonadarche occurs in a variety of disorders of sexual maturation, including premature adrenarche (onset of pubic or axillary hair before age 8), chronic adrenal insufficiency, true precocious puberty (when the onset is before age 6), primary hypogonadism, isolated gonadotropin deficiency and anorexia nervosa.

**Adrenal Estrogen:**

The adrenal cortex secretes estrone and estradiol, but the amounts are minimal compared with those Adrenal secreted by the ovary. Most adrenal estrogens are derived indirectly from peripheral conversion of androstenedione, mainly in adipose tissue and muscle.

The origin of plasma estrogen has been clarified by such studies. In normal women most plasma estradiol is derived by direct secretion from the ovary. There is little, if any, estradiol formed testosterone by extraglandular conversion. On the other hand, little estrone is formed by direct ovarian secretion, and most estrone in plasma originates from extraglandular conversion of androstenedione and to a minor extents, from estradiol. The primary site of extraglandular
aromatization of androstenedione to estrone is in adipose tissue, and the rate is influenced by age, liver function, and thyroid function.

The importance of extragonadal estrogen formation is increased in a variety of clinical conditions. The formation of estrogens by the placenta depends on androgens secreted by the fetal adrenal gland and, to a lesser extent, on androgens from the maternal circulation. In nonpregnant premenopausal women, estrone is formed from androstenedione secreted by the ovary and adrenal. In menopausal women the ovarian formation of androstenedione is negligible, but considerable amounts of estrone are formed by extraglandular conversion of androstenedione secreted by the adrenals. An increase in estrogen formation occurs with aging and obesity, sufficient to produce endometrial hyperplasia and breeding in monopausal women. In premenopausal women, ovarian tumors or polycystic ovarian syndrome (PCOS) can cause increased secretion of androstenedione and secondary increases in estrone formation. In such women the amount of extraglandular production of estrogen can interfere with normal feedback mechanisms and produce disturbance of the ovarian cycle.

The presence of abnormal hair growth (hirsutism) or masculinization (virilization) implies excessive androgen, principally testosterone. The women serves. Excessive testosterone may result from increased secretion by the adrenal or ovary and increased extraglandular production from androstenedione secreted by the ovary or adrenal, as her own bioassay, and the rate of production of testosterone
Toxicological implication of steroid biosynthetic mechanisms.

The enzymes of steroid biosynthesis are the targets for range of different toxicants (Raven and Hinson 1996) the adrenal cortex also has a well reported ability to activate toxins, an effect mediated by both the cytochrome p-450 enzymes of steroid biosynthesis as well as other enzymes. The zone specific effects of many toxicants may reflect the differences in the enzymes expressed by the different zones; e.g. 17-hydroxylase, an enzyme strongly implicated in the actions of several adrenal toxicants, including spironolactole, is expressed in the highest concentration in the zona fasciculata, with negligible activity in the zona glomerolusa, explaining why the effects of this toxin are specific to the zona fasciculata. This also explains why many toxicants such as spironolactone, are specific (Colby 1996), with effects being apparent in guinea pigs, dogs and humans but not in rats and mice the effects of adrenocortical enzymes on the activation of adrenal toxicants have been extensively studied by Colbi and coworkers.

Cholesterol

Cholesterol is derived from the Greek word meaning “solid bile” and it may be defined like hard waxy material that is found in many food items including milk, cheese, eggs, butter, ghee, fish, beef, pork, chicken and goat meat. It is naturally occurring fat-like substance with a complex chemical formulae and is used to build cells and make hormones (Gupta 1996). Some amount of cholesterol is
necessary for the proper functioning of sex hormones and vitamin -d metabolism in the body. In young children, cholesterol is required for the development of brain cells. The membranes of many cell require a minimum quantity of cholesterol for healthy functioning.

**Sources and metabolism of cholesterol:**

Cholesterol in the blood comes from two main sources:-

1. the cholesterol ingested from outside, that is taken in daily diet. An average vegetarian consumes in between 200-400 mg. of cholesterol daily, while non vegetarian usually consumes between 400-600 mg of cholesterol.

2. A large part of cholesterol in the blood comes from cholesterol production within the liver. It has been observed that if the oral intake of cholesterol through blood is reduced then the liver tends to produce a little extra cholesterol, at least, during the initial few weeks.

The mechanism of metabolism of cholesterol is rather complex and involves complicated pathways, receptors (site on the cell that receive a particular substance), and enzyme action.

The body handles cholesterol that is ingested through different food items in its own way. Cholesterol itself, can not be dissolved in blood or water, like many food particles, after absorption from the intestine, the dietary fat and
cholesterol are transferred to the liver for further metabolic action. In the liver, cholesterol combines with water soluble substances called apolipoproteins and phospholipids to form chylomicrons are form of complex particles called lipoproteins, that is fats combined with proteins (Guyton and Hall, 1996).

**Toxicological implications of lipoprotein uptake and storage:**

The uptake of HDL and LDL has obvious implications for the actions of toxins on the adrenal cortex: as the adrenocortical cells have the specific uptake mechanism for lipoproteins, any lipophilic toxin associated with circulating lipoproteins will also be taken up by the adrenal. Indeed it has been demonstrated that adrenocortical cells do have a remarkable capacity for taking up and concentrating certain toxins, including PCB metabolites (Brandt 1987), methacrylonitrile (Ahmed et al. 1996), DDT metabolites (Lund et al. 1988), toluene (Pyykko et al. 1977) and 1-aminobenzotriazole (Town et al. 1993).

**Adrenal insufficiency:**

**Primary adrenal insufficiency**

Primary adrenal insufficiency, which can be acute or chronic, may be caused by the anatomic destruction of the gland. This destruction can have various causes, including tuberculosis (TB) or fungal infection, other diseases infiltrating the adrenal glands, and hemorrhage. However, the most frequent cause is idiopathic
atrophy, which is probably autoimmune in origin.

Primary adrenal insufficiency also may be caused by metabolic failure (eg, insufficient hormone production). This failure may be a result of congenital adrenal hyperplasia, enzyme inhibitors (eg, metyrapone), or cytotoxic agents (eg, mitotane). Primary adrenocortical insufficiency is rare and it occurs at any age. The male-to-female ratio is 1:1.

**Secondary adrenal insufficiency**

Secondary adrenal insufficiency may be caused by hypopituitarism due to hypothalamic-pituitary disease, or it may result from suppression of the hypothalamic-pituitary axis by exogenous steroids or endogenous steroids (ie, tumor).

Secondary adrenocortical insufficiency is relatively common. Extensive therapeutic use of steroids has greatly contributed to increased incidence.

**Acute adrenocortical insufficiency**

Adrenal crisis may result from an acute exacerbation of chronic insufficiency, usually caused by sepsis or surgical stress. Acute adrenal insufficiency also can be caused by adrenal hemorrhage (eg, usually septicemia-induced Waterhouse-Friderichsen syndrome [fulminant meningococcemia]) and anticoagulation complications. Steroid withdrawal is the most common cause of acute adrenocortical insufficiency, and it almost exclusively causes a
glucocorticoid deficiency.

Although primary adrenocortical insufficiency affects men and women equally, women are affected 2 to 3 times more often by the ideopathic autoimmune form of adrenal insufficiency. A considerable work has been done on adrenal toxicity and gonadal toxicity in relation to bodymetabolism and function status in animal kingdom but, the relationship between gonad and adrenal remained relatively unexplored so I choose this topic i.e. “studies on some gonad-adrenal endocrine profiles of Wistar albino-rat with special reference to toxicant”

**Review of Literature**

A number of chemicals such as pesticides are known to interfere with the endocrine system and thereby impair fertility and the development of animals and possibly of humans.

The adrenal glands have many features which render them particularly susceptible to toxicological damage. Indeed of all endocrine tissues susceptible to toxicologic lesions, the adrenal cortex is the tissue most often affected **Rebelin(1984)**. The adrenal gland secrete hormones which are important components of the physiological stress response, and when adrenal function is compromised there are likely to be severe consequences for individual exposed to stressful stimuli. several previous reviews have described the variety of chemical toxicants that have been found to cause functional or histological lesions in
the adrenal gland (Rebelin 1984; Szaba and Lippe 1989; Colby 1996)

Some toxicants that interact with stress- mediated hypothalamic –pituitary and sympathoadrenal systems might influence puberty. Such indirect effects could also result from toxicants that influence appetite or nutrient absorption. An example of a toxicant that affects puberty in rodents is the insecticide DDT. Exposure of neonatal rats to DDT causes major changes in neuroendocrine functions, including early puberty and a syndrome of delayed onset, persistent estrus in association with a polyfollicular ovarian status (Heinrichs et al;1971). This permanent reproductive impairment in female rodents resembles the neonatal masculinization of the hypothalamus and the polyfollicular, anovulatory ovarian syndrome caused by exogeneous steroids (Gorski 1971 Mobbs et al 1984) Whether such effects of DDT are limited to a critical period during development is unknown.

Environmental stress delays puberty in laboratory rodents. The onset of puberty is associated with achievement of a critical body size and fat content (Frisch, 1980, Tanner 1981).

Exposure of rodents just before or after birth to estrogens and other steroids has profound effects on adult reproductive functions that can be manifested at puberty or can induce precocious cessation of fertile cycles (de-
layed anovulatory syndrome). The mechanism by which DDT causes persistent estrus may involve an estrogenic action, since DDT has uterotrophic effect (Bitman et al. 1968) and also can bind to cytosolic E2 receptors (Robison et al. 1985). This example shows how environmental toxicants can interact with neuroendocrine maturation.

Adrenal insufficiency in human may delay puberty (Boyar et al. 1973) but this association is not found in all cases (Reiter and Grumbach, 1982), adrenallectomy of rats delays puberty, whereas corticoid replacement restores he normal onset (Ramley, 1978). There is general agreement that adrenal steroids are not obligatory for maturation of hypothalamic controls over the gonad in either sex.

Prenatal exposure to synthetic hormone diethyloestilbestrol (DES) is the best documented example of toxic influence on development female reproductive track anomalies of vagina cervix uterus fallopian and mezonephric duct remnants found in humans with hysterosalpingography and usually detected after reproductive maturity seems to originated from disturbances in embryonic development (Newbold, Melachian 1982). Among affected these abnormalities structure may comprise reproductive performance (Herbest et al., 1980). Whether prenatal exposure to DES also alters the hypothalamus or pituitary is being investigated (Mayer and Bahiburg et. al., 1985).
Corticotrophin (ACTH) has an important role in maintaining the normal structure of the adrenal cortex. In the absence of ACTH, following hypophysectomy. For example, the inner zones become atrophied, and the normal arrangement of cells in the zona fasciculata is lost (Vinson et al., 1992). When ACTH is administered, there is a general increase in the size of the gland, with hypertrophy and hyperplasia of the inner zone cells. ACTH also causes a widening of the vascular sinusoids, and a chronic exposure to high ACTH levels, a breakdown of the adrenal vasculature of the zona glomerulosa is relatively unaffected by removal of ACTH stimulation, although following prolonged ACTH treatment, there is a decrease in size of this zone, as glomerulosa cells are transformed into fasciculata type cells (McDougall et al., 1980; Pudney et al., 1984).

There are many reports of the effects of toxins on adrenal morphology. The effects vary with different agents used: hyperplasia is seen with aflatoxin and stilboestrol, acute disseminated necrosis is seen with carbon tetrachloride, acute necrosis with dimethylbenzanthracene, cortical adenoma with urathane and striking spironolactone bodies are seen with prolonged use of this diuretic (Neville et al. 1982; Ribelin 1984). Some more recently described effects of toxins on adrenal morphology include cortical necrosis induced by dimethylacetamide in mice (Valentine et al., 1997).

The recently developed ACAT (Acyl-CoA cholesterol acyltransferase)
inhibitors developed to lower blood cholesterol levels have been reported to cause adrenal atrophy/necrosis in cynomolgus monkies (Reindel et al., 1994), dog (Wolfgang et al., 1995) and rabbit (Matsuo et al., 1996). vacuolation of the zona fasciculata in rats treated with chlorpyrifos, an organophosphate insecticide (Breslin et al., 1996).

Review of toxicity to gonads-

Testicular toxicity can be caused by any chemical, physical or biological agent that alters physiological control processes that affects the normal functioning of the testes. Much work has been done on toxicity to male reproductive system by using different toxicants.

Agricultural chemicals implicated in male reproductive toxicity include DDT (O-chlorodiphenyltrichloroethane), epichlorohydrine, ethylene dibromide, kepone and the dioxins (Whorton et al., 1997). DBCP, a nematoside widely used in agriculture, is a testicular toxicant and induces hypergonadotropic hypogonadism (Mattison 1983; Popashnic et al., 1987). DDT, a commonly used pesticide and its metabolites have oestrogenic effect in males by blocking androgen receptors.

Many anti microbial (e.g. tetracycline derivatives, sulphadiazine, nitrofurantoin) impair spermatogenesis and spermatozoal functions(
Ericsson et al., 1967; Schlegel et al., 1991) methachloretamine, extensively used as a nitrogen mustard during the second world war, causes spermatogenic arrest (Spitz 1948). Many common cytotoxic agents cause a dose dependent progressive decrease in sperm count, leading to azoospermia (Meistrich 1982).

Numerous pharmacological agents and many clinically approved drugs affect the testes especially the higher doses (Margalioth et al., 1985) administration of gonadotropin releasing hormone agonists and related analogues leads to supression of gonadotropins and spermatogenesis. Ketoconazole, an anti-fungal agent inhibits testosterone biosynthesis primarily by inhibiting the activities of steroidogenic enzymes in Leydig cells without any direct effect at the pituitary level (Sikka et al., 1985; Bhasin et al., 1986).

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidents, commonly known as reactive oxygen species, which belongs to the class of free radicals. Chronic disease states, ageing, toxin exposure and exposure to many types of environmental contaminants can enhance this oxidative process and cause gonadal damage (Sikka et al., 1995). Nitric oxide along with super oxide radicals induces endothelial cell injury (Beckman et al., 1990), which may resulting testicular disfunctions.
Cellular damage is theoretically the result of an improper balance between ROS generation and intrinsic scavenging activities. Adequate levels of super oxide dismutase (SOD) catalase and probably glutathion peroxidase and reductase normally maintain the free radical scavanging potenilia in testes; this balance can be referred to as oxidative stress status and its assessment may play a critical role in monitoring testicular toxicity and infertility (Sikka 1997). The mechanism of female reproductive toxicity vary for example a toxicant might mimic the action of naturally occurring reproductive hormones. Many chemically unrelated compounds – including stilbene derivatives (Example DES), industrial chemicals (Example PCBs), and pesticides (Example DDT) exhibit estrogenic activity in bioassays, and thus have the potential to alter the normal estrogen feedback relationship between the gonad and the brain and so disrupt ovulation in a manner analogous to that of oral contraceptives. Environmental agents might alter hormone synthesis, storage, release, transport or metabolism. E.g. compounds with estrogenic activity can cause luteolysis and inhibit the production of progesterone, as a consequence of these and other considerations (such as alteration in fallopian tube and endometrium functions),

Malathion has also been associated with birth defects in domestic and laboratory animals. In rabbits, malathion crosses the placenta and acts on the central nervous system injection of malathion into the yolk sac of chicken eggs
caused reduced growth and weakening of a leg bone increased production of insulin reduced chick weights, reduced hatch, short legs, bleached down, nerve damage two to six weeks after hatchingsparse plumage, limb shortening, growth reduction, and beak defectsReproductive EffectsJuvenile male rats exposed to daily doses of malathion had decreased numbers of sperm-forming cells In two rat teratology studies, maternal exposure to malathion reduced pup weights, increased the incidence of hemorrhagic spots on the backs of pups, and decreased weight gain of the mothersDoses of 50 and 100 mg/kg/day of malathion caused pregnant rabbits to have reduced maternal weight gain and greater increases of fetal resorptions (dead fetuses absorbed into the mother, not aborted); statistically significant increases in maternal deaths occurred at all dosesA two generationstudy of male and female rats exposed to malathion yielded offspring that weighed less than the controls, and had increased susceptibility to ring-tail disease In sheep, malathion exposure of pregnant ewes resulted in an increase in aborted fetuses, still births, low birth weight babies. Longer duration and earlier initiation of malathion exposure resulted in more severe problems.

Grover et. al. worked on effect of metronidazole on spermatogenesis and FSH, LH and testosterone levels of prepubertal rats. Metronidazole, a 5-nitroimidazole drug has been reported to decrease testicular weight and epididymal spermatid counts and causes abnormal sperm morphology with degen-
eration of seminiferous tubules with 6 weeks treatment of metronidazole (400 mg/Kg, Day). In contrast to DNA flow cytometry (FCM), the histological and gravimetric parameters do not allow a rapid, sensitive objective and multiparameteric evaluation of reproductive toxicants on spermatogenesis. Moreover, the exact mechanisms for such an effect are not entirely clear. This study assess the effects of intraperitoneal administration of metronidazole 400 mg/Kg daily for 30 days on testicular germ cell changes assessed by DNA and hormone levels of testosterone, FSH and LH in pre-pubertal rats. A significant reduction in the haploid cell population in metronidazole treated groups as compare to saline treated controls was observed. The mean serum FSH, LH and testosterone value were also lowered in treated animals. Thus, the spermitotoxic effects of metronidazole were probably mediated by decrease in the circulating hormones responsible for spermitogenesis.

(Sarkar et al., 2002) studied the effects of chronic sub-lethal doses (7-14 mg/Kg, a day for 15 days) of quinalphos were evaluated in adult male rats for changes in testicular morphology, circulatory concentration of hormone (LH, FSH, prolactin and testosterone), activities of acetylcholinesterase (AChE) and angiotensin converting enzyme (ACE) as well as metabolism of biogenic amines (dopamine, noradrenaline and 5-hydroxytryptamine) in the hypothalamus and pituitary. Sub-lethal chronic administration of quinalphos resulted in
decrease testicular mass and AChE activity in central as well as peripheral organs; increased serum LH, FSH, prolactin and testosterone concentration; decreased pituitary or increased testicular ACE activity; severe disruption of spermatogenesis with increase in doses of pesticide; and no significant effects on dopamine, nor adrenaline or 5-HT concentrations in the hypothalamus pituitary. Administration of oestradiol (50mg/rat a day) during pesticide treatment resulted in; a significant decrease in the mass of the testis and accessory sex organs; decreases in serum LH, FSH, testosterone concentration; an increase in prolactin concentration; and a decrease in dopamine or an increase in noradrenaline and 5-HT in the hypothalamus or pituitary. Oestradiol had a mark effect: in pesticide-treated animals, the pesticide effects were significantly reversed. This indicates that in pesticide toxicity, the hypothalamo-pituitary-gonadal axis is operational. Since many of the observed pesticide effects could be inhibited by oestrodiol, it is suggested that the pesticides acts directly on the gonadotrophins. In conclusion, quinalphos decreases fertility in adult male rats by affecting the pituitary gonadotrophins.

One important insight is that toxicants which perturb ovarian function may pose a particular problem for female health because the general health of the female is closely linked to reproductive health, which in turn is directly linked to ovarian function.
Ovarian cysts were observed in both control and DES-treated mice at two months of age. However, in control animals, the ovarian bursa was the site of cyst formation whereas, in the Des-treated animals, cyst appeared to originate from prominent mesonephric remnants. The irregular lining epithelium of the cysts resembled cells of mesonephric origin rather than ovarian surface epithelium.

In vitro studies of DES-exposed ovary suggests that steroidogenesis is impaired (Haney et al. 1981, 1984).

Female Sprague-Dawley rats (70 days old) were initiated with a single dose of 175 mg/kg diethylnitrosamine (DEN) intraperitoneally in saline vehicle whereas control groups received saline only. Treatment with DEN initiation and TCDD promotion significantly increased the incidence of ovarian tumours. The highest incidence of ovarian tumours developed in 20% rats initiated with DEN and promoted with TCDD for 60 weeks, whereas, no ovarian tumours developed in controls. Using RT-PCR, immunohistochemistry and in situ hybridization, the AhR was localized to oocytes and granulosa and theca cells of growing follicals, surface epithelial cells and rete tubule and epithelial cells in ovaries from adult control Sprague-Dawley rats and in the neoplastic cells in the ovarian tumours.
DES and MXC illustrate that compounds that activate the estrogen receptor have a profound impact on the developing reproductive and endocrine systems, which indirectly impacts ovarian function, but may also have subtle direct effects on ovarian development and adult ovarian function. Perhaps, this occurs because the classical estrogen receptor is needed only late in follicular development, as illustrated by the estrogen-receptor deficient mouse phenotype (Couse et al., 1995). DEHP illustrates a direct acting ovarian toxicant that suppresses the ability of the granulosa cells to synthesise estrogen.

The realization that environmental chemicals can impaired reproductive health through receptor that mediated pathways has understandably caused considerable public health concern. However, the impact that endocrine disruptors have on human health is still determined by the same factors that govern the impact of other environmental toxicants.

In the rat, sexual behaviour is thought to be irreversibly organised during the perinatal period by exposure of the brain to testosterone and its metabolites, which bind to androgene and estrogen receptors within the CNS (McEwan et al., 1977; Baum, 1979). In the neonate, the local formation of DHT by 5a-reductase and 17-b-oestradiol by aromatase within the HPOA is essential for the normal sexual differentiation of the male CNS. This occurs within a critical window of neonatal development and reliance on testosterone supplied by the fetal
Leydig cells. Compounds which inhibit the binding of testosterone or oestradiol in the HPOA region of the brain have disruptive effects on sexual behaviour. If this occurs during neonatal development then irreversible changes in sexual behaviour of the male are likely to occur (Steindeck et. al., 1970; Booth 1978; Hirsch et. al., 1986, 1987).

Ethanol exposure during this critical period significantly inhibits Leydig cell steroidogenesis by inhibiting 17α-hydroxylase activity and decreases plasma testosterone levels (Kelce, et. al., 1990), reduce to fertility secondary to effects on sexual behaviour have been seen in male rats administered fenarimol, a pyrimidine carbinol agricultural fungicide (Hirsch et. al., 1986). The suggested mechanism of action was through inhibition of aromatase activity within the CNS, there by preventing the bio-synthesis of oestradiol and the development of estrogene receptors in the brain during the early postnatal period (Hirsch et. al., 1987).

Lead toxicity affects many tissues but its effects on male reproductive function appear to result from both peripheral and centrally mediated effects (Chapin and Williams 1989). Feeding rats with leadacetate results in lowered LH and testosterone levels and decreased elongating spermatids. In lead-treated rats, stimulation of the Leydig cells by exogenous LH showed normal testosterone release, and stimulation of pituitaries with GnRH showed higher than normal
LH release. However, administration of naloxone, which increases LH levels in control animals through CNS pathways, was ineffective in lead-treated rats, suggesting and effect at the level of the hypothalamus or other controlling neuronal pathways (Sokol and Madding et al., 1985, 1987).

In vitro and in vivo experiments with Sprague-Dawley rats showed that the three organophosphate insecticide tested depressed endogenous cortisol synthesis and blocked corticosteroidogenesis in response to ACTH and cAMP stimulation of a suspension of adrenal cells. Pregnenolone stimulation of adrenal cells was not inhibited at the insecticide concentration which blocked the ACTH and cAMP stimulation of corticosteroidogenesis. It was concluded rat insecticides act beyond the site of action of ACTH and at or beyond the level of cAMP metabolism and prior to the metabolism of pregnenolone.

Effect of malathion, an organophosphate compound on plasma concentration luteinizing hormone (LH) and testosterone (T4) was studied in adult male rats. A single s.c. administration of malathion (23 mg/kg, 1/50th LD 50 dose) significantly (p<0.05) reduced plasma concentration of both LH and T4 at 24, 36 and 48 h post-treatment. Prior administration of human chronic gonadotropins (hCG, 50 IU) for two days at 24 h interval protected the malathion induce hormonal changes. These results suggest that malathion induce inhibition of testicular steroidogenesis may be due to its cholinergic activity influ-
cing central mechanisms involved in LH secretion and/or release. Malathion [0,0-dimethyl S(1,2-dicarboethoxyethyl)phosphorodithioate] shows adverse effects on reproductive endocrine function and performance in various species have been reported. Single or repeated exposure of malathion affect the steroidogenesis and fertility in cows and ewes with or without any significant inhibition of plasma acetylcholinesterase activity. Although, the influence of indirectly acting cholinergic agonists like organophosphorus compound on anterior pituitary function in little understood, there were evidences that cholinergic system is involved in secretion/release of pituitary gonadotrophins. The effect of single injection of malathion on plasma luteinizing hormone (LH) and testosteron(T4) concentrations in rats and also whether human chorionocgonadotrophy(hcG)treatment could restore the impaired steroidogenesis.

The realization that environmental chemicals can impair reproductive health through receptor mediated pathways has understandably caused considerable public health concern however, the impact that endocrine disrupters have on human health is still determined by the same factors that govern the impact of other environmental toxicants.