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Xenobiotics with estrogen or antiandrogen action - disruptors of the male reproductive system

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Abstract: The environmental and life-style changes associated with developing industry and agriculture, especially the exposure to endocrine disrupting chemicals (xenobiotics), are considered as causes of the increasing incidence of male reproductive system disorders. Most of the xenobiotics, which harmfully influence the male reproductive system, reveal estrogen-like (xenoestrogens) or anti-androgenic activity. Recent data have revealed physiological roles of estrogens in the male, however, there are evidences that estrogen-like substances may lead to many undesirable symptoms in the male i.e. gonadal dysgenesis, genital malformations, cryptorchidism, decreased fertility potential and testicular neoplastic changes. The number of xenoestrogens is still growing in the environment, whereas the mechanisms of their action are still not exactly known. They can be harmful not only to the present but potentially also to the next generations.

Keywords: Endocrine disrupting chemicals, xenobiotics, xenoestrogens, gonadal dysgenesis, genital malformations, cryptorchidism, testicular germ cell tumours, infertility

Abbreviations

AFP alphafetoprotein
BPA bisphenol A
CIS testicular carcinoma in situ
DBP di(n-butyl) phthalate
DDT dichloro-difenylo-trichloroethan
DES diethylstilbestrol

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ER estrogen receptor
ERKO mice without ER
FSH follicle stimulating hormone
GCT testicular germ cell tumours
HDL high density lipoprotein cholesterol
hg haplogroup
HPTE 2,2-bis(phydroxyphenyl)-1,1,1-trichloroethane
LDL low density lipoprotein cholesterol
LH luteinizing hormone
MAPK mitogen-activated protein kinase
MC metoxychlor
OP octylphenol
p,p'-DDE 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
PRL prolactin
PVC polyvinyl chloride
SHBG sex hormone binding globuline
SXR steroid/xenobiotic receptor
TDS testicular dysgenesis syndrome
TNF tumour necrosis factor

1 Estrogens in the male

Estrogens have been classically considered as the main hormones in the female reproduction but contemporary there is a growing number of evidences that estrogens play a role in the development and physiology of the male reproductive tract [1, 2]. Estrogen may play a regulatory role for the initiation of spermatogenesis as a stimulatory factor for spermatogonia multiplication and as a factor which enhances FSH effects on the initiation of spermatogenesis [3–6]. Clinical studies demonstrated a precociously complete spermatogenesis in boys with pseudo-precocious puberty because of Leydig cell hyperplasia associated with hypersecretion of estradiol [7, 8]. Recently, the presence of functional estrogen receptor (ER) has been demonstrated in differentiating male external genitalia, what indicates a possible novel role of estrogens in the regulation of the development of these sex structures [9, 10]. It has been established that estrogen influences growth and maturation of bones, and is necessary to achieve epiphyseal closure and peak bone mass in the human male [11–13]. Estrogens have effects throughout the brain. Regulation of the serotoninergic system appears to be linked to the presence of estrogen-sensitive neurons in the midbrain raphe, whereas the influence of estrogens on cholinergic function involves induction of choline acetyltransferase and acetylcholinesterase, according to a sexually dimorphic pattern. During the period of development, when testosterone is elevated in the male neonate, aromatase and ERs are transiently expressed in the hippocampus, what strongly suggests that this pathway is involved in the masculinization of hippocampal structure and function [14]. Endogenous estrogens may have positive [15]

as well as negative influence on the circulatory system in men [16].

ER is the most ancient among the vertebrate steroid receptors. Steroid receptors are extremely ancient and widespread, having diversified from a primordial gene before the origin of bilaterally symmetric animals. This gene was lost in the lineage leading to arthropods and nematodes and became independent of hormone regulation in the mollusk Aplysia lineage [17]. The evolution of sex steroid receptors involved changes in protein-protein interactions as well as ligand recognition [18]. The sequences of the hormone-binding domain in fish steroid receptors have diverged more from their orthologs in land vertebrates than amphibian and mammalian orthologs from each other. This sequence divergence correlates with differences in ligand specificity between fish and land vertebrate steroid receptors. Steroids that are active in fish or were active in ancestral vertebrates may still have activity in mammals, but in different context. Recognition of androgens and estrogens by an ancestral androgen receptor or ER may explain findings for cross-recognition of steroids by the mammalian androgen receptors and ERs [18, 19]. This indicates that phylogeny needs to be considered in evaluating the effects of environmental chemicals on steroid-responsive processes.

ERs are abundant throughout the male reproductive tract. In many vertebrate species both ERα and ERβ have been found. ERα is primarily localized in the epithelium of efferent ductules, that are a series of tubules connecting rete testis to the epididymis. Here, the function of ERα is the regulation of the rete testis fluid reabsorption to concentrate sperm prior to entering the epididymis [20]. ERβ is more widely distributed in the male tract than ERα and has also strong reactivity in efferent ductules, similar to ERα. ERα is not present in the seminiferous epithelium, while ERβ is found in germ and Sertoli cells [21]. The third isoform ERγ has been cloned in year 2000 from the teleost fish *Micropogonias undulates* [22]. ERγ binds 17β-estradiol with high affinity. Three forms of ER in teleost fish suggests that the estrogen signal may be distributed in the complicated networks in the fish but probably also in other vertebrate species what needs further investigation.

2 Male reproductive system anomalies – fact or fiction in the epidemiological data

An increase in the incidence of male reproductive system anomalies has been shown in several reports from different countries after the Second World War. In EUROCAT Website Database, an European network of population-based registry for the epidemiologic surveillance of congenital anomalies (started in 1979) more than 1.5 million births surveyed per year are registered [23]. 43 registries in 20 countries are included what means that 29% of European birth population is covered. In years 1980–2003 the mean prevalence of the genital anomalies called intermediate sex (true hermaphroditism and male or female pseudohermaphroditism) was 0.77/10000 births/year. The highest value was observed in 1982 – 1.13/10000 and the lowest in 2003 – 0.55/10000. The mean prevalence of hypospadias in the same time was 7.96/10000/year. The highest value was noted in 1990-

1991 – 8.88/10000 and the lowest in 2003 – 5.99/10000. In the EUROCAT data from the end of 1990's the decrease in the incidence of genital malformations has been observed in all European countries. Some other estimates show that the prevalence of hypospadias in Europe and USA has been increasing from 1960s to 1980s, but these trends might not be continuing [24, 25]. Nevertheless, numbers from the Polish Registry of Congenital Malformations, which was introduced in 1997 and comprised 52.9% of the total area of the country, are significantly higher than those published in the EUROCAT. The incidence of reproductive tract anomalies (without cryptorchidism) is 23.6/10000 births/year. The most frequent anomaly of the male reproductive system is hypospadias - 40.5/10000 male neonates/year. Disturbances of male genitalia differentiation constitute about 13% of all inherited anomalies in Poland and are located in the 3^{rd} place after malformations of circulatory and bone systems [26]. Moreover, most estimates of hypospadias prevalence in Europe and USA range up to 30/10000 births/year, with approximately 75% of cases being glandular and coronal [25, 27, 28], what indicates much higher prevalence than it is given in the EUROCAT. From a Finnish study [29] it appeared that the higher prevalence of hypospadias in Finland was probably due to the improvement of the ascertainment by the congenital anomaly register, while surgical discharge records showed a stable prevalence. According to these divergences, the epidemiological data may be no longer clear and reliable. However, regardless of the problems with the estimation and interpretation of the frequency of genital malformations, the absence of increase in the other congenital anomalies is observed on the contrary to the data on genital anomalies. The real existence of the problems with the health of male reproductive system may be strengthen by the reports that not only the frequency of congenital anomalies, but also the related disturbances such as cryptorchidism, epididymal anomalies, testicular germ cell tumours (GCT) and the decrease in the number of sperms in ejaculate, appeared to be rising at the same time [30, 31].

The great majority of the published data are in favour of an increase in the incidence of cryptorchidism over recent decades in Europe and North America [32]. In 1999 Paulozzi [25] performed a meta-analysis to compare the incidence of cryptorchidism from several industrialized countries, using data collected by the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS). In USA a continuous increase (1970-1994) was observed. In Canada the incidence of cryptorchidism increased with an incidence rate growing from 140/10000/year to 240/10000/year (1974-1990). In the Western European countries the incidence of cryptorchidism increased not so much: in France from 80/10000/year to 120/10000/year and similarly in England, while in Norway was stable with about 150/10000/year.

The incidence of GCT in European men (1.1-11.8/100000/year) depends on the country and is the highest in Denmark. The incidence of GCT increased rapidly in all European countries after the Second World War, by 2-3.5% annually in Nordic countries and by about 5% in Poland and Germany [33–36]. The rising trend is more pronounced for ages below 30. The age-standardized incidence of GCT doubles every 15 to 25 years [35].

The meta-analysis of Carlsen *et al.* [30] revealed a significant decrease in sperm

concentration (from 113 mln/ml to 66 mln/ml) and semen volume (from 3.4 ml to 2.75 ml) over the period of 1938-1990. In a French study 2.1% per year decrease in sperm concentration during years 1973–1992 was observed [37]. Furthermore, in the latter study the percentage of motile spermatozoa and spermatozoa with normal morphology also decreased significantly, whereas semen volume remained unchanged. The decreasing trend in semen quality was not observed in Finland, where the semen concentration was found to be the highest in Europe (124 mln/ml) [38]. The differences in semen quality might depend on the geographical region but also on the methodology of its evaluation [39–41]. Nevertheless, Swan *et al.* [40] after reanalysis of the studies by Carlsen *et al.* and after addition of the data from other studies found that the reported trends for years 1938-1990 are also seen in data for 1934-1996.

Skakkebaek *et al.* [31] have suggested that the male reproductive problems may be one entity with the same etiology. They proposed the existence of a new clinical syndrome – a testicular dysgenesis syndrome (TDS), which comprises the wide range of developmental retardations of the testes, including disturbed organogenesis and cryptorchidism. The authors proposed that male infertility (oligo- and azoospermia) and testicular GCT resulted also from TDS. The justification is that in all of these disturbances immature seminal tubules with undifferentiated Sertoli cells, Sertoli-cell-only tubules, intratubular microliths and tubules containing *carcinoma in situ* (CIS) were detected. The structural lesions of the testes may suggest that the functional lesions of Sertoli and Leydig cells also exist and are the cause of reproductive system anomalies in fetal period of life and spermatogenic failure in adulthood. Moreover, disturbed internal gonadal milieu may promote neoplastic changes of germ cells. All the disorders included in TDS are the risk factors for GCT development. The coexistence of intersex condition with GCT has been reported since years but only the scarce clinical material was available. In our own study on the large clinical material (40 patients with gonadal dysgenesis and 6 patients with true hermaphroditism) CIS was detected in 55% of cases with gonadal dysgenesis, 10% with androgen insensitivity, 17% with unilateral GCT and 33% of patients with undescended testes [42]. The incidence of neoplastic lesions was the highest in patients with gonadal dysgenesis (65%). Among them the highest risk occurred in patients with partial (91%) and mixed gonadal dysgenesis (77%) [43], while rarely in gonads less developed (pure gonadal dysgenesis - 23%), what indicates that neoplastic changes appear in a relatively well-developed gonadal structure. It appeared also that testis structure by itself predisposed to the initiation of germ cell neoplasia. In our other epidemiological approach, the presence of neoplastic changes of germ cells appeared to be independent on the structural and numerical aberrations of sex chromosomes which were generally considered to be a cause of gonadal dysgenesis and GCT. It is observed that the risk of GCT is higher in brothers than in sons of the patients with GCT what indicates less genetic, but more environmental influences [44, 45].

3 Reasons of the increase in the frequency of reproductive abnormalities

The environmental and life-style changes associated with developing industry and agriculture are considered as the reasons of the increase in the frequency of reproductive abnormalities. In Poland the incidence of GCT is about 6-times higher in the industrially developed regions in comparison to the ecologically pure [33]. Some of the authors suggested the detrimental effects of the exposure to synthetic progestins used from 1960's for the maintenance of pregnancy with the high risk of miscarriage, as contraceptives or as a part of chemicals used in pregnancy tests [46, 47] but a meta-analysis performed by Raman-Wilms [48] found no association between such exposure and male external genitalia malformations.

The exposure to environmental endocrine disrupting chemicals (xenobiotics) is suggested to be a reason of the increase in the frequency of reproductive abnormalities [49–51]. This hypothesis is confirmed by the growing number of reports on the male reproductive system anomalies in wild living animals, including snails, fish, amphibians, birds and mammals [52–55]. Reproductive problems even in polar bears indicate that contamination with endocrine disrupters may be a global problem [55]. Most of the xenobiotics which harmfully influence the male reproductive system reveal at least partly the action of natural estrogens, thus they are called xenoestrogens, and often simultaneously anti-androgen action [24, 34, 56].

4 Xenoestrogens - endocrine disrupting chemicals with estrogenic effects

There are evidences that estrogen-like substances, both in the male and female, may lead to many undesirable symptoms. The wide phylogenetic distribution of estradiol production and the estrogen signal recognition system in the animal kingdom suggests the possibility that all animals are sensitive to estrogens, whether endogenous or environmental [51]. The variability in ER isoforms and tissue specific distribution gives the oportunity to exert different actions in the organisms by xenoestrogens which in some aspects may mimic the action of natural sex hormone 17β-estradiol or bind to ER, despite their different chemical structure. Although ER has not been found in invertebrate species, there are evidences that estrogen-like chemicals can alter their metabolism and development. The explanation for this can be the existence of a large and still growing number of receptors with unknown function or ligand, which are called orphan receptors. One of them is steroid/xenobiotic receptor (SXR) which recognizes many xenobiotics and activates different responses [51].

4.1 Agricultural xenoestrogens and anti-androgens

An example of the reproductive abnormalities, which are the result of xenoestrogen action, in wild living animals may be the demasculinization of alligators in Lake Apopka at Florida [57, 58]. These reptiles have decreased phallus size, abnormal gonads and altered sex hormone levels due to the endocrine-disrupting effects of DDT (dichlorodifenylo-trichloroethan) and the related organochlorine pesticides. DDT is the first and the most known pesticide, which can act as an estrogen agonist [59] or anti-androgen [60]. The major and persistent DDT metabolite is p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), which has little ability to bind to ER, but inhibits androgen binding to the androgen receptor, androgen-induced transcriptional activity, and androgen action in developing, pubertal and adult male rats [61]. DDT was discovered in 1939 by Paul Müller as a very effective insecticide. It quickly became the most widely used pesticide in the world. However, in the 1960's, it was discovered that DDT cause birth defects in animals and humans. DDT is now banned in many countries, but it is still used in some developing regions to prevent malaria and other tropical diseases by killing mosquitos and other disease-carrying insects. In North-Eastern Brazil, one of the poorest regions, where 50% of the population lives in favellas, mosquito and rodent proliferation is extended. DDT and other insecticides are widely used by the inhabitants, in addition to broad agricultural pesticide application. The incidence of genital malformations in male newborns is dramatically high in this region – 94/10000/year [62].

Metoxychlor (MC) was developed as a replacement for the banned pesticide DDT. After *in vivo* administration, it is metabolized in the liver to 2,2-bis(phydroxyphenyl)-1,1,1-trichloroethane (HPTE), which is an active agent. Both MC and HPTE exhibit weak estrogenic and anti-androgenic activities. Recently, Murono *et al.* [63] revealed that daily *in vivo* administration of MC (200 mg/kg) leads to the decline of serum testosterone and dehydroepiandrosterone levels and decline of seminal vesicle weights in young adult male rats. *Ex vivo* Leydig cell basal testosterone formation and Leydig cell P450 cholesterol side-chain cleavage activity declined, supporting previous *in vitro* studies which demonstrated the sensitivity of this step to MC.

Pesticide use has increased 50-fold since 1950, and 2.5 mln tons of industrial pesticides are now used each year. The most widely used pesticides today are nonpersistent organophosphates, including glyphosphate, which is currently the world's most used herbicide. The pesticides shown to have estrogenic or anti-androgenic properties include chlordecone, dieldrin, vinclozin, endosulfan, toxaphene and linuron [64].

Several investigators have shown striking differences in semen quality, GCT rate, cryptorchidism and hypospadias between Denmark and Finland [35, 38, 65, 66]. It was demonstrated that the concentrations of pesticides in the human breast milk were significantly lower in Finland than in Denmark, suggesting Danish children to be most exposed than Finnish children [67]. DDT and some other pesticides are biodegradated in a long-time period, so they can be accumulated in the organisms and influence upon the next generations. Developmental abnormalities may be the result from *in ovo* or *utero*

exposure, presumably of maternal origin [68].

4.2 Industrial xenoestrogens and anti-androgens

Not only pesticides but also different chemicals for everyday use and environmental pollutants have been shown to possess estrogen-like and anti-androgenic bioactivity i.e. detergents, organic solvents, paints, combustion products, heavy metals. Phthalates refers to a class of additives that are used in some plastic products made with polyvinyl chloride (PVC) to make the material soft and flexible. It is used for the production of toys, medical devices, flooring, wires, rainwears, food packaging and additives to cosmetics, clothing, medicines [69]. Phthalate metabolites have been detected in the urine, saliva, serum, seminal fluid and breast milk [70–74]. They exert estrogenic and antiandrogenic effects in rodents and humans [75]. The mechanism of phthalate action has not yet been completely elucidated. They interfere with Leydig cell function and act predominantly as anti-androgens. In utero exposure of male rats to di(n-butyl) phthalate (DBP) induces hypospadias, cryptorchidism, decreased sperm count and testosterone level in adulthood. Fetal DBP exposure causes also changes in testes similar to TDS in humans i.e. areas of malformed seminiferous cords/tubules with intratubular Leydig cells, multinucleated gonocytes, immature Sertoli cells, and abnormal aggregation of Leydig cells [76–78] .

Widely used is bisphenol A (BPA), a monomer of polycarbonate plastics and constituent of epoxy and polystyrene resins, used in the food packaging industry and dentistry [79]. Detectable amounts of BPA were found in food cans and human saliva after treatment with dental sealants [80, 81]. The next is octylphenol (OP), a constituent of alkylphenol polyethoxylates that are used as surfactants in detergents, paints and herbicides [82]. Alkylphenols are degradated in plants to stable products which can accumulate within internal organs of fish and birds, and thus pass through the food chain to humans. BPA and OP can act as the weak estrogens and influence the reproductive health [83].

Numerous published reports have linked exposure to heavy metals such as lead, cadmium and mercury with male infertility. Battery workers intoxicated with these metals have defects of spermatogenesis [84]. It was found that the exposure to inorganic lead produced inhibition of intratesticular testosterone synthesis in animals and in men [85]. Treatment with lead and cadmium in animals was associated with the increase in apoptosis of spermatogenic cells [86, 87]. Recently, it has been suggested that cadmium is a new environmental estrogen because it can mimic some effects of estradiol [88, 89].

4.3 Medical xenoestrogens and anti-androgens

Much more higher estrogenic effects has diethylstilbestrol (DES), a synthetic potent estrogen, which was widely used in medicine as postcoital contraception, the maintenance of high risk pregnancy with imminent miscarriage, suppression of lactation, estrogen replacement therapy and prostate cancer therapy. In agriculture DES was used to stimulate growth of chicken and cattle. DES and its metabolites were present in the meat

and excreted to ecosystem. Exposure *in utero* to DES resulted in increased prevalence of male urogenital tract abnormalities, cryptorchidism, GCT and reduced fertility in men [90, 92, 93].

There are also some studies on the environmental contamination with the estrogenic components of commonly used oral contraceptive 17α-ethinyl estradiol [94, 95]. Estrogens and their glucuronides have been found in municipal sewage, surface and waste waters in industrial regions.

Neonatal treatment of male rats with high doses of DES or ethinyl estradiol induces a range of developmental abnormalities such as inhibition of Sertoli cell proliferation [96], suppression of Leydig cell development and function [97], abnormal development of the rete testis [93], efferent ducts [98], epididymis and vas deferens [93, 99], seminal vesicles [100] and prostate [101, 102]. Additionally, DES suppresses expression of the androgen receptor [93].

4.4 Phytoestrogens

Chemicals with some estrogenic activity are present in the environment not only as pollutants resulting from the developing civilisation, but also as chemicals naturally produced by plants. Some of them are used in the traditional medicine and kitchen, mainly in Asia. Plant compounds with estrogen-like biological activity are called phytoestrogens. There are three main phytoestrogen classes: isoflavones, coumestans and lignans. Genistein, which belongs to the isoflavones group, is of the greatest interest at present. Isoflavones are found in fruits and vegetables, but predominantly in leguminous plants and are especially abundant in soybean. Phytoestrogens may exert both estrogenic and anti-estrogenic effects on metabolism, depending on several factors, including their concentration, the concentrations of endogenous estrogens and individual characteristics such as gender and reproductive status in women [103].

It was found that isoflavones exert some benefit effects: anti-cancerogenic (inhibit tumor cell proliferation, angiogenesis and cell cycle progression), anti-cardiovascular disease (decrease of total cholesterol and LDL cholesterol, increase of HDL cholesterol, improve endothelial function), anti-osteoporotic (increase bone mineral density) and anti-menopausal syndromes (decrease frequency of hot flushes) [104, 105]. Currently the increase in plant-based foods consumption, especially soy products, is recommended to increase fibre and antioxidant intakes, replacing sources of saturated fat and cholesterol in the diet.

Nevertheless, phytoestrogens can exert also adverse effects, mainly on male and female reproductive systems. These effects were first observed in sheep, which became infertile during feeding with subterranean clover [106]. It was found that chronic exposure of spermatozoa to high doses of genistein could be associated with infertility problems through suppression/inhibition of acrosome reaction and sperm motility [107]. Paris *et al.* [108] have demonstrated for the first time that phytoestrogens (among others genistein) exhibit some antiandrogenic action.

4.5 Mycoestrogens and anti-androgens

The dietary estrogens originate not only from plants and meat of animals treated with hormones but also from food contamination with moulds. Consumption of cereal contaminated with *Fusarium sp* has been associated with estrogenic effects in poultry and livestock [109, 110]. Zearalenone, a fungal mycotoxin produced by *Fusarium,* binds to ER [111]. Recently, it was demonstrated that mycoestrogens (zearalenone and its metabolites) are nearly as potent as flutamide, an antiandrogenic medication [108]. These indicate that prenatal exposure to phyto/mycoestrogens via vegetarian diet during pregnancy may alter male fetal sex differentiation and further reproductive ability.

Vinclozolin is a fungicide that has been reported to have anti-androgenic effects in male rats, but probably may also exert its effects by involving additional steroid-signaling pathways. It has morphological effects similar to those of medroxyprogesterone acetate, feminizing males and leading to hypospadias. At the molecular level, vinclozolin up-regulated ERα and progesterone receptor mRNA in male rats, effects seen also with exposure to the synthetic estrogen, ethinyl estradiol [112]. Anway *et al.* [113] have recently demonstrated that transient exposure at the time of male sex determination to vinclozolin can induce an apparent epigenetic transgenerational phenotype with reduced spermatogenic capacity.

5 Mechanisms of xenobiotics' action

5.1 Receptor binding

Endocrine disrupting chemicals can bind to the tissue receptor sites of the specific hormone and act as agonists or antagonist of the hormones. The receptor may behave as a signal integration unit and collect information from growth factors, other nuclear receptors and series of chaperone and co-regulator proteins [51]. The response depends on the convergence of activating ligands and cellular signals, the multiple receptor isoforms, contributions of co-activators and co-repressors [114]. Despite their different chemical structure xenobiotics can influence the same receptors. For instance, cadmium has been demonstrated to activate ERα by interacting with the ligand-binding domain of the receptor [89].

5.2 Changes in hormonal metabolism

Xenobiotics can interfere with the hormone biosynthesis, secretion, metabolism/elimination and alter hormone homeostasis. It was revealed that chronic subcutaneous administration of pesticide OP to adult male rats decreased serum FSH, LH and testosterone levels, and increased PRL [115, 116]. The authors observed shrinkage of the testes and male accessory sex organs. Sperm numbers were reduced and the evaluation of sperm morphology revealed marked increase in the proportions of head and tail abnormalities. When

OP was administered to the male rats in the drinking water no significant alterations were observed in mean serum LH, FSH and testosterone levels and reproductive organ weights but only an increase in epididymal sperm with tail abnormalities [115]. The highest doses decreased epididymal sperm number. Khurana *et al.* [117] found that exposure of newborn rats to OP or BPA results in delayed and sustained hyperprolactinemia and increased expression of ERs in the anterior pituitary of males, whereas the hypothalamic ERs were less responsive. Although BPA and OP can act as the weak estrogens [83], it was reported by Steinmetz *et al.* [118] that the estrogenic effects of BPA were higher *in vivo* than expected from *in vitro* studies. Xenoestrogens do not bind to the sex hormone binding globuline (SHBG) what may explain their large bioeffects even in low concentrations.

5.3 Genomic imprinting

It was established also that xenobiotics directly or through related pathways play a role in programming or imprinting genes involved in cell proliferation, differentiation or survival. A key event in establishing the pattern of gene expression in a cell is the methylation or demethylation of regulatory elements of the gene. The persistent change in gene expression is called genomic imprinting. It is accomplished in epigenetic fashion and leads to the silencing of a gene from one parent. The epigenetic changes in gene function that occur without a change in the sequence of nuclear DNA may be heritable. Descendants of the cell in which the gene was turned on or off will inherit this activity even if the original stimulus for gene-activation is no longer present. Diffusion of the gene's product to other cells can also make the heritable characteristic spread.

Little is known about the role of estrogens in gene imprinting. Nevertheless, it was found that estrogen-associated signalling pathways may contribute to DNA methylation or demethylation [51]. Developmental exposure to estrogens resulted in the persistent overexpression of lactotransferrin in the uterus of female mouse [119, 120]. It has been presented also that developmental exposure to DES can perturb normal uterine development by affecting genetic pathways governing uterine differentiation [121]. DDT binds to and activate ERs, but it was found recently that DDT targets also non-ER pathways [122]. DDT and its metabolites stimulate activator protein-1 (AP-1)-mediated gene expression through the p38 mitogen-activated protein kinase (MAPK) cascade and in this way induces apoptosis and the expression of the death ligand TNF-α. McLachlan [51] suggests that cellular imprinting by estrogens may arise through the mechanisms: 1) directly imprinting of the gene probably by the DNA methylation, leading to persistent genetic change, and 2) alteration of signalling pathways at key points in cell differentiation, resulting in altered gene expression.

5.4 Genetic polymorphism

Genetic polymorphism means the occurrence together in the same population of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone. Genetic aberrations or polymorphism may predispose to augmented effects by environmental factors. In the study of Danish population it was found that a specific Y chromosome haplogroup (hg26) is significantly over-represented in men with reduced sperm counts [123]. It is suggested that the factors encoded by genes on this class of Y chromosome may be particularly susceptible to environmental pollutions that cause reproductive disturbances in Denmark. Ogata *et al.* [124] suggest that homozygosity for the specific ERα haplotype rises incidence of cryptorchidism in response to endocrine disruptors.

5.5 Influence of xenoestrogens on the male fetus and infant

It was believed for more than 50 years that prenatal exposure to estrogens interferes with the normal development of testes and reproductive organs in males, resulting in the reduced fertility in adulthood [125]. In 1988 Kula was the first, who described the positive, stimulative influence on the spermatogenesis during the prepubertal period of life in rats [3]. The investigation of mice without ER (ERKO mice) revealed that the male mice had normally developed reproductive system and were fertile but the progressing damage of spermatogensis and fertility with the age was observed as the result of inadequate epididymal function [126]. ERα is abundant in the efferent ductules of the testis and epididymis during fetal life in humans, suggesting a major role of estrogen in the development and function of male reproductive structures [127].

Nielsen *et al.* [128] found that ERα may be involved in the development of Leydig and peritubular cells. Nevertheless, the enhanced estrogenic signalling can suppress the biosynthesis of insulin-like 3 hormone (Insl3) by Leydig cells, which is necessary for the development of gubernaculum, and by attenuating the production of androgens, necessary for the regression of the cranial suspensory ligament [124]. Defects of Insl3 action cause cryptorchidism in male mice, while over-expression in female mice causes ovarian descent. Recently, Ferlin and Foresta [129] demonstrated that serum level of Insl3 is decreased in adult men with infertility caused by severe inherited testicular damage, reflecting the functional status of the Leydig cells.

Xenoestrogens do not bind to SHBG and also to alphafetoprotein (AFP), which is present in the fetal circulation and acts against (inactivate) endogenous estrogens influence on the male fetus. Thus, the male fetus is not protected against the exogenous estrogen-like chemicals revealing the enhanced estrogenic action [130]. Andersson and Skakkebaek [50] warn about the xenobiotic levels in meat from hormone-treated animals, which are probably too high and not safe for prepubertal children and human fetuses. Wisniewski *et al.* [131, 132] found that perinatal (gestation and lactation) exposure of female rats and mice to "natural" phytoestrogen – genistein, in doses common in human di-

ets, altered masculinisation (smaller anogenital distance and testis size, delayed preputial separation) and decreased testosterone concentration in adulthood. Perinatal exposure to genistein caused also long-term dysfunction in sexual behaviour (less intention to mount, intromit and ejaculate). Moreover, aggressive behaviours were decreased, while defensive increased. These results give warning in the face of popularity of soy infant formulas. The authors suggest that the influence of isoflavone exposure during early childhood on reproductive and behavioural health in boys and men should be considered. Phytoestrogens isoflavones bind ERα and ERβ and alter the transcription of estrogen-responsive genes [83]. Phyto- and mycoestrogens can act also as antiandrogens by lowering the androgen receptor nuclear translocation and the intranuclear cluster formation induced by androgens [108].

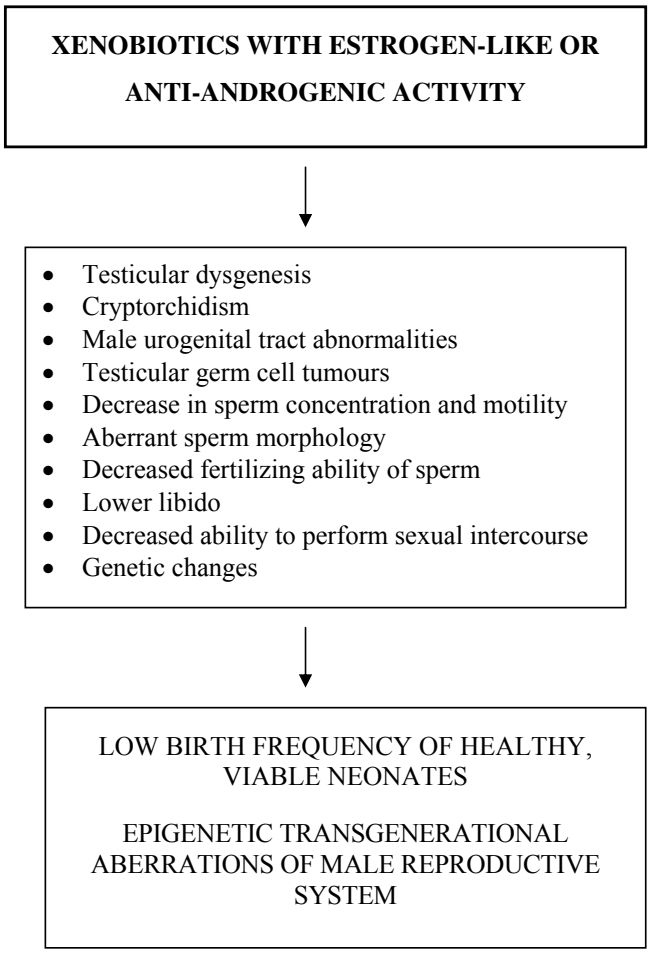

Fig. 1 Detrimental effects of xenobiotics with estrogen-like or anti-androgenic action in the male.

Exposure to exogenous estrogens may diminish production of the fetal pituitary gonadotropin FSH by negative feedback mechanism. Lower FSH serum levels may result in decreased rate of Sertoli cells proliferation and disturbed synthesis of substances which create intratesticular milieu and control the activity of germ, Leydig and peritubular cells

within testes. Disturbed biosynthesis of Sertoli cells products may result in disorders of the differentation of male reproductive system, cryptorchidism, formation of testicular neoplastic changes and furthermore infertility [49]. It has been hypothesized that in dysgenetic gonads of intersex individuals with Y chromosome material in the karyotype Sertoli cells have impaired function but their activity may be enough strong to prevent the primordial germ cells from entering meiosis, as it occurs in the ovary. There may be, however, too few or too poor functioning Sertoli cells to stimulate adequate differentiation of gonocytes into spermatogonia. The primordial germ cells may not have clear signals to differentiate either into female or into mature male germ cells and they therefore kept their fetal characteristics as multipotential gonocytes (stem cell potential).

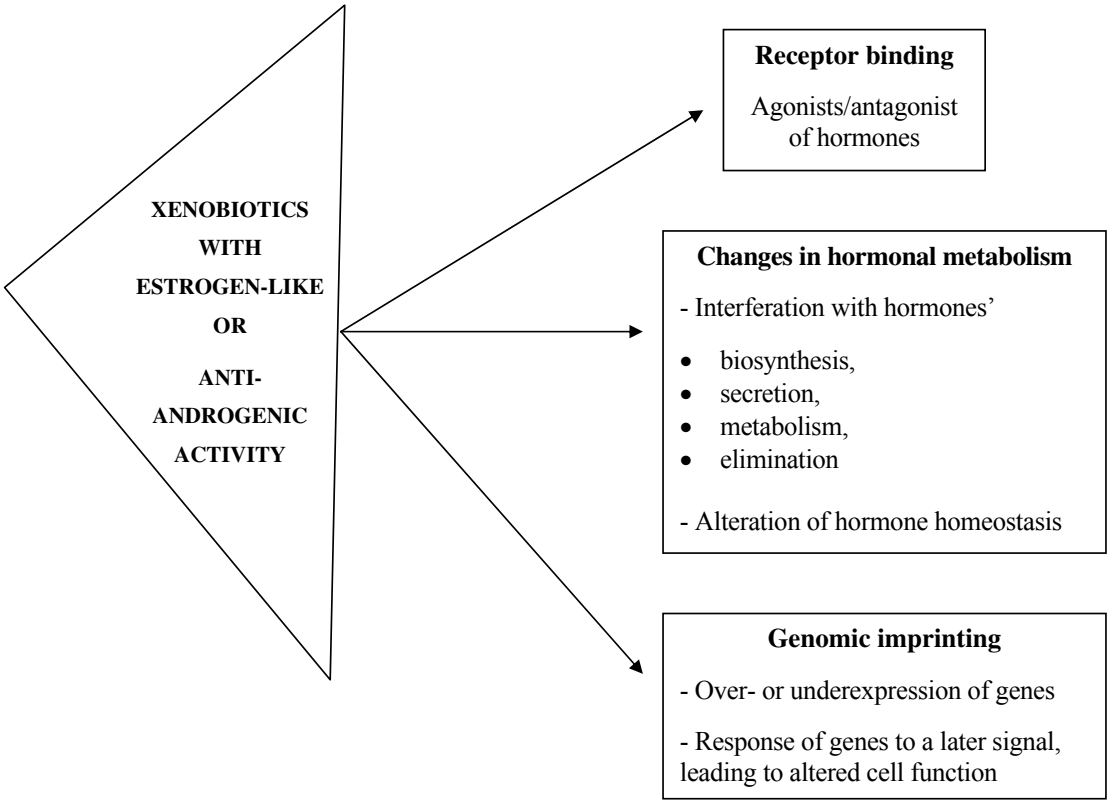

Fig. 2 Known mechanisms of xenobiotics' action..

6 Summary and conclusions

Although physiologic action of estrogen in the male reproductive system, bones, brain and other organs is of vital importance, it is necessary to realize that most of endocrine disruptors reveal estrogen-like activity, which is not under the control of physiological mechanisms, and/or anti-androgenic activity, and due to these are harmful to male reproductive system, both during development and in adulthood (Fig. 1). The number of these substances is still growing, what depends on the development of civilisation,

whereas the mechanisms of their action are still not exactly known (Fig. 2). Potentially, endocrine disruptors may have also adverse effects in the subsequent generations. Therefore the responsibility lays on the contemporary human population to control the environmental pollution and try to diminish their influence on the nature.

References

[1] K.S. Korach: "Insights from the study of animals lacking functional estrogen receptor", *Science,* Vol. 266, (1994), pp. 1524–1527.

[2] S. Carreau, S. Lambard, C. Delalande et al.: "Aromatase expression and role of estrogens in male gonad: a review", *Reprod. Biol. Endocrinol.,* Vol. 1, (2003), p. 35.

[3] K. Kula: "Induction of precocious maturation of spermatogenesis in infant rats by human menopausal gonadotropin and inhibition by simultaneus administration of gonadotropins and testosterone", *Endocrinol.,* Vol. 122, (1988), pp. 32–39.

[4] F.J.P. Ebling, A.N. Brooks, A.S. Cronin et al.: "Estrogenic induction of spermatogenesis in the hypogonadal mouse", *Endocrinol.,* Vol. 141, (2000), pp. 2861–2869.

[5] K. Kula, R. Walczak-Jędrzejowska, J. Słowikowska-Hilczer et al.: "Estradiol enhances the stimulatory effect of FSH on testicular maturation and contributes to precocious initiation of spermatogenesis", *Mol. Cell. Endocrinol.,* Vol. 178, (2001), pp. 89–97.

[6] R. Walczak-Jędrzejowska, J. Słowikowska-Hilczer, E. Oszukowska et al.: "Initiation of spermatogenesis may require simultaneous action of two steroid hormones: estradiol and testosterone", In: *International Proceedings*, Medimond, 2005, pp. 91–95.

[7] J. Słowikowska-Hilczer, M. Metera, R. Walczak et al.: "Spermatogenesis in boys with precocious puberty and excessive secretion of testosterone and estradiol", *Ginekol. Pol.,* Vol. 66, (1995), pp. 71–76.

[8] K. Kula, J. Słowikowska-Hilczer, T.E. Romer et al.: "Precocious maturation of the testis in the increased secretion of estradiol and testosterone by Leydig cells", *Pediatr. Pol.,* Vol. 3, (1996), pp. 269–273.

[9] S. Jesmin, C. N. Mowa, N. Matsuda et al.: "Evidence for a potential role of estrogen in the penis: detection of estrogen receptor –alpha and –beta messenger ribonucleic acid and protein", *Endocrinol.,* Vol. 143, (2002), pp. 4764–4774.

[10] C. Crescioli, M. Maggi, G. B. Vanelli et al.: "Expression of functional estrogen receptors in human fetal male external genitalia", *J. Clin. Endocrinol. Metab.*, Vol. 88, (2003), pp. 1815–1824.

[11] C. Carani, K. Qin, M. Simoni *et al.*: "Effect of testosterone and estradiol in a man with aromatase deficiency", *New Engl. J. Med.,* Vol. 337, (1997), pp. 91-95.

[12] M. Faustini-Fustini, V. Rochira and C. Carani: "Oestrogen deficiency in men: where are we today?", *Europ. J. Endocrinol.*, Vol. 140, (1999), pp. 111–129.

[13] V. Rochira, A. Balestrieri, M. Faustini-Fustini *et al.*: "Role of estrogen on bone in the human male: insights from the natural models of congenital estrogen deficiency", *Mol. Cell Endocrinol.*, Vol. 178, (2001), pp. 215–220.

[14] B.S. McEwen and S.E. Alves: **"**Estrogen actions in the central nervous system",

Endocrine Rev., Vol. 20, (1999), pp. 279-307.

[15] M.M. Grumbach and R.J. Auchus: "Estrogen: Consequences and implications of human mutations in synthesis and action", *J. Clin. Endocrinol. Metab.*, Vol. 84, (1999), pp. 4677–4694.

[16] J.K. Wranicz, I. Cygankiewicz, M. Rosiak et al.: "The relationship between sex hormones and lipid profile in men with coronary artery disease", *Int. J. Cardiol.*, Vol. 101, (2005), pp. 105–110.

[17] J.W. Thornton, E. Need and D. Crews: "Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling", *Science,* Vol. 301, (2003), pp. 1714–1717.

[18] M.E. Baker: "Adrenal and sex steroid receptor evolution: environmental implications", *J. Mol. Endocrinol.*, Vol. 26, (2001), pp. 119–125.

[19] S. Yeh, H. Miyamoto, H. Shima et al.: "From estrogen to androgen receptor: A new pathway for sex hormones in prostate", *PNAS,* Vol. 95, (1998), pp. 5527–5532.

[20] R.A. Hess, Q. Zhou and R. Nie: "The role of estrogens in the endocrine and paracrine regulation of the efferent ductules, epididymis and vas deferens", In: *The epididymis: from molecules to clinical practice*, Kluwer Academic/Plenum Publishers, New York, 2002, pp. 317–338.

[21] P.T. Saunders, R.M. Sharpe, K. Williams et al.: "Differential expression of oestrogen receptor alpha and beta proteins in the testes and male reproductive system of human and non-human primates", *Mol. Hum. Reprod.,* Vol. 7, (2001), pp. 497–503.

[22] M.B. Hawkins, J.W. Thornton, D. Crews et al.: "Identification of a third distinct estrogen receptor and reclassification of estrogen receptors in teleolosts", *Proc. Natl. Acad. Sci. USA,* Vol. 97, (2000), pp. 10751–10756.

[23] *EUROCAT Website Database*, www.biomedicalweb.biz/eurocat, 2005.

[24] J. Toppari, J.C. Larsen, P. Christiansen et al.: "Male reproductive health and environmental xenoestrogens", *Environ. Health Perspect.,* Vol. 104, (1996), pp. 741–803.

[25] L.J. Paulozzi: "International trends in rates of hypospadias and cryptorchidism", *Environ. Health Perspect.,* Vol. 107, (1999), pp. 297–302.

[26] A. Latos-Bieleńska, A. Materna-Kiryluk, M.R. Krawczyński et al.: "Genital malformations in Poland – data from the Polish Registry of Congenital Malformations", *Horm. Res.,* Vol. 5, (1999), p. 78.

[27] L.J. Paulozzi, J.D. Erickson and R.J. Jackson: "Hypospadias trends in two US surveillance systems", *Pediatr,* Vol. 5, (1997), pp. 831–834.

[28] F.H. Pierik, A. Burdof, I. M.R. Nijma et al.: "A high hypospadias rate in the Netherlands", *Hum. Reprod.,* Vol. 17, (2002), pp. 1112–1115.

[29] M. Aho, A.M. Koivisto, T.L.J. Tannela et al.: "Is the incidence of hypospadias increasing? Analysis of Finnish Hospital discharge data 1970-1994", *Environ. Health Perspect.,* Vol. 108, (2000), pp. 463–465.

[30] E. Carlsen, A. Giwercman, N. Keiding et al.. "Evidence for decreasing quality of semen during past 50 years", *Br. Med. J.,* Vol. 30, (1992), pp. 609–613.

[31] N.E. Skakkebaek, E. Rajpert-DeMeyts and K.M. Main: "Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects",

Hum. Reprod., Vol. 16, (2001), pp. 972–978.

[32] P.F. Thonneau, P. Candia and R. Mieusset: "Cryptorchidism: Incidence, risk factors, and potential role of environment; an update", *J. Androl.,* Vol. 24, (2003), pp. 155–162.

[33] J. Didkowska, U. Wojciechowska, W. Tarkowski et al.: *Cancer in Poland in 1999,* Centrum Onkologii, Instytut im. M. Skłodowskiej-Curie, Zakład Epidemiologii i Prewencji Nowotworów, Warszawa, 2002.

[34] J. Toppari, J. C. Larsen, P. Christiansen et al.: *Male reproductive health and environmental chemicals with estrogenic effects*, Danish Environmental Protection Agency, Copenhagen, 1995.

[35] H. Adami, R. Bergstrom, M. Mohner et al.: "Testicular cancer in nine Northern European countries", *Int. J. Cancer*, Vol. 59, (1994), pp. 33–38.

[36] H. Møller and H. Evans: "Epidemiology of gonadal germ cell cancer in males and females", *APMIS,* Vol. 111, (2003), pp. 43–46.

[37] J. Auger, J.M. Kunstmann, F. Czyglik et al.: "Decline in semen quality among fertile men in Paris during the past 20 years", *New Engl. J. Med.*, Vol. 332, (1995), pp. 281–285.

[38] J. Suominen and M. Vierula: "Semen quality of Finnish men", *Br. Med. J.,* Vol. 306, (1993), p. 1579

[39] S.H. Swan, E.P. Elkon and L. Fenster: "Have sperm densities declined? A reanalysis of global trend data", *Environ. Health Perspect.,* Vol. 105, (1997), pp. 1228–1232.

[40] S.H. Swan, E.P. Elkon and L. Fenster: "The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996", *Environ. Health Perspect* ., Vol. 108, (2000), pp. 961–966.

[41] N. Jőrgensen, E. Carlsen, I. Nermoen et al.: "East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland", *Hum. Reprod.,* Vol. 17, (2002), pp. 2199–2208.

[42] J.Słowikowska-Hilczer, R. Walczak-Jędrzejowska and K. Kula: "Immunohistochemical diagnosis of preinvasive germ cell cancer of the testis", *Folia Histochem. Cytobiol.,* Vol. 39, (2001), pp. 67–72.

[43] J. Słowikowska-Hilczer, T.E. Romer and K. Kula: "Neoplastic potential of germ cells in relation to disturbances of gonadal organogenesis and changes in karyotype", *J. Androl.,* Vol. 24, (2003), pp. 270–278.

[44] E. Rapley, G. P. Crockford, D. Teare et al.: "Localisation to Xq27 of a susceptibility gene for testicular germ-cell tumours", *Nat. Genet.,* Vol. 24, (2000), pp. 197–200.

[45] E. Rapley, G.P. Crockford, D.F. Easton et al.: "Localisation of susceptibility genes for familial germ cell tumour", *APMIS,* Vol. 111, (2003), pp. 128–135.

[46] A.S. Goldman and A.M. Bongiovanni: "Induced genital anomalies", *Ann. NY Acad. Sci.,* Vol. 142, (1967), pp. 755–767.

[47] A. Czeizel, J. Toth and E. Erodi: "Aetiological studies of hypospadias in Hungary", *Hum. Hered.,* Vol. 38, (1979), pp. 45–50.

[48] L. Raman-Wilms, A. Lin-in Tseng, S. Wighardt et al.: "Fetal genital effects of first-

trimester sex hormone exposure: a meta-analysis", *Obstet. Gynecol.,* Vol. 85, (1995), pp. 141–149.

[49] Skakkebaek N.E., E. Rajpert-deMeyts, N. Jőrgensen et al.: "Germ cell cancer and disorders of spermatogenesis: An environmental connection?", *APMIS* , Vol. 106, (1998), pp. 3–12.

[50] A.M. Andersson and N.E. Skakkebaek: "Exposure to exogenous estrogens in food: possible impact on human development and health", *Eur. J. Endocrinol.,* Vol. 140, (1999), pp. 477–485.

[51] J.A. McLachlan: "Environmental Signaling: what embryos and evolution teach us about endocrine disrupting chemicals", *Endocr. Rev.,* Vol. 22, (2001), pp. 319–341.

[52] T.E. Bryan, R.P. Gildersleeve and R.P. Wiard: "Exposure of Japanese quail embryos to o,p'-DDT has long-term effects on reproductive behaviors, hematology, and feather morphology", *Teratology,* Vol. 39, (1989), pp. 525–535.

[53] E. Willingham and D. Crews: "Sex reversal effects on environmentally relevant xenobiotic concentrations on the red-eared slider turtle, a species with temperature-dependent sex determination", *Gen. Comp. Endocrinol* ., Vol. 113, (1999), pp. 429–435.

[54] J. Oehlmann, U. Schulte-Oehlmann, M. Tillmann et al.: "Effects of endocrine disruptors on prosobranch snails (*Mollusca: Gastropoda*) in the laboratory. I. Bisphenol A and octylphenol as xeno-estrogens", *Ecotoxicol.,* Vol. 9, (2000), pp. 383–397.

[55] J.G. Vos, E. Dybing, H.A. Greim et al.: "Health effects on endocrine-disrupting chemicals on wildlife, with special reference to the European situation", *Crit. Rev. Toxicol.*, Vol. 30, (2000), pp. 71–133.

[56] R.M. Blair, H. Fang, W.S. Branham et al.: "The estrogen receptor relative binding affinities of 188 natural and xenochemicals:structural diversity of ligands", *Toxicol. Sci.,* Vol. 54, (2000), pp. 138–153.

[57] L.J. Guillette Jr, T.S. Gross, G.R. Masson et al.: "Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida", *Environ. Health Perspect.,* Vol. 102, (1994), pp. 681–688.

[58] L.J. Guillette Jr, A.R. Woodward, D.A. Crain et al.: "Plasma steroid concentrations and male phallus in juvenile alligators from seven Florida lakes", *Gen. Comp. Endocrinol.,* Vol. 116, (1999), pp. 356–372.

[59] R.M. Welch and A.H. Conney: "Estrogenic action of DDT and its analogs", *Toxicol. Appl. Pharmacol.,* Vol. 14, (1969), pp. 358–367.

[60] S.C. Laws, S.A. Carey and W.R. Kelce: "Differential effects of environmental toxicants on steroid receptor binding", *Toxicologist,* Vol. 15, (1995), p. 294.

[61] W.R. Kelce, C.R. Stone, S.C. Laws et al.: "Persistent DDT metabolite p,p"-DDE is a potent androgen receptor antagonist", *Nature*, Vol. 375, (1995), pp. 581–585.

[62] D. Sampaio, J.B. Neto, F. Audran et al. : "Explosive rise in malformations of newborn male genitalia in Northeastern Brazil: Consequences of a polluted environment?", *Horm. Res.,* Vol. 64, (2005), pp. 127–128.

[63] E.P.Murono, R.C. Derk and Y. Akgul: "In vivo exposure of young adult male rats to methoxychlor reduces serum testosterone levels and ex vivo Leydig cell testosterone formation and cholesterol side-chain cleavage activity", *Reprod. Toxicol.*, Vol. 21, (2006), pp. 148–153.

[64] G.A. LeBlanc, L.J. Bain and V.S. Wilson: "Pesticides: multiple mechanisms of demasculinization", *Mol. Cell. Endocrinol.,* Vol. 126, (1997), pp. 1–5.

[65] N.E. Skakkebaek and N. Keiding: "Changes in semen and the testis", *Br. Med. J.,* Vol. 309, (1994), pp. 1316–1317.

[66] K. A. Boisen, M. Kaleva, K. M. Main et al.: "Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries", *Lancet*, Vol. 363, (2004), pp. 1250–1251.

[67] I.N. Damgaard, H. Virtanen, M. Kaleva et al.: "Pesticides in Danish and Finnish breast milk samples", *Horm. Res.,* Vol. 64, (2005), p. 128.

[68] M.R. Milnes, D.S. Bermudez, T.A. Bryan et al.: "Altered neonatal and endocrine function in Alligators mississipiensis a.ssociated with contaminated environment", *Biol. Reprod.*, Vol. 73, (2005), pp. 1004–1010.

[69] T. Schettler: "Human exposure to phthalates via consumer products", *Int. J. Androl.,* Vol. 29, (2006), pp. 134–139.

[70] H.M. Koch, H. Drexler and J. Angerer: "An estimation of the daily intake of di(2-ethylhexyl) phthalate (DEHP) and other phthalates in the general population", *Int. J. Hyg. Environ. Health,* Vol. 206, (2003), pp. 77–83.

[71] G.K. Mortensen, K.M. Main, A.M. Andersson et al.: "Determinations of phthalate monoesters in human breast milk, consumer milk and infant formula by tandem mass spectrometry (LC/MC/MS)", *Analyt. Bioanalyt. Chem.,* Vol. 382, (2005), pp. 1084–1092.

[72] M.J. Silva, J.A. Reidy, A.R. Herbert et al.: "Detection of phthalate metabolites in human amniotic fluid", *Bull. Environ. Contam. Toxicol.*, Vol. 72, (2004b), pp. 1226–1231.

[73] M.J. Silva, J.A. Reidy, E. Samandar et al.: "Detection of phthalate metabolites in human saliva", *Arch. Toxicol.,* Vol. 79, (2005), pp. 647–652.

[74] MJ Silva, D.B. Barr, J.A. Reidy et al.: "Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000", *Environ. Health Persp.,* Vol. 112, (2004a), pp. 331–338.

[75] D. Seidlova-Wuttke, H. Jarry and W. Wuttke: "Pure estrogenic effect of benzophenone-2 (BP2) but not of bisphenol A (BPA) and dibytylphthalate (DBP) in uterus, vagina and bone", *Toxicol.,* Vol. 205, (2004), pp. 103–112.

[76] J.S. Fisher, S. Macpherson, N. Marchettiet al.: "Human"testicular dysgenesis syndrome": a possible model using in-utero exposure of the rat to dibutyl phthalate", *Hum. Reprod.,* Vol. 18, (2003), pp. 1–13.

[77] I.K. Mahood, N. Hallmark, C. McKinnell et al.: "Abnormal Leydig cell aggregation in the fetal testis of rats exposed to di(n-butyl) phthalate and its possible role in testicular dysgenesis", *Endocrinol.,* Vol. 146, (2005), pp. 613–623.

[78] I.K. Mahood, C. McKinnell, H. Walker et al.: "Cellular origins of testicular dysgenesis in rats exposed in utero to di(n-butyl) phthalate", *Int. J. Androl.,* Vol. 29, (2006), pp. 148–154.

[79] N. Ben-Jonathan and R. Steinmetz: "Xenoestrogens: the emerging story of bisphenol A", *Trend. Endocrinol. Metab.,* Vol. 9, (1998), pp. 124–128.

[80] J.A. Brotons, M.F. Olea-Serrano, M. Villalobos et al.: "Xenoestrogens released from lacquer coatings in food cans", *Environ. Health Perspect.*, Vol. 103, (1995), pp. 608–612.

[81] N. Olea, R. Pulgar, P. Perez et al.: "Estrogenicity of resin-based composites and sealants used in dentistry", *Environ. Health Perspect.,* Vol. 104, (1996), pp. 298–305.

[82] C.E. Purdom, P.A. Hardiman, V.J. Bye et al.: "Estrogenic effects of effluents from sewage treatment works", *Chem. Ecol.,* Vol. 8, (1994), pp. 275–285.

[83] G.G.J.M. Kuiper, B. Carlsson, K. Grandien et al.: "Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β", *Endocrinol.,* Vol. 138, (1997), pp. 863–870.

[84] S.E. Chia, C.N. Ong, S.T. Lee et al.: "Blood concentrations of lead, cadmium, mercury, zinc, and copper and human semen parameters", *Arch. Androl.,* Vol. 29, (1992), pp. 177–183.

[85] M. Rodamilans, M.J. Martinez-Osaba, J. To-Figueras et al.: "Inhibition of intratesticular testosterone synthesis by inorganic lead", *Toxicoll. Lett.,* Vol. 42, (1988), pp. 285–290.

[86] W. Awara, N. El-Ashmawy, S. Nassar et al.: "Fas signalling system mediates cadmium-induced testicular apoptosis in rats. A protective role of selenium", In: *Andrology of the 21st Century*, Medimond, 2001.

[87] C. Millan, R. Z. Sokol, Q. Shu et al.: *Lead induces epigenetic modification of rat testicular gene expression: a DNA microarray study*, Andrology of the 21st Century, Medimond, 2001.

[88] P. Garcia-Morales, M. Saceda, N. Kenney et al.: "Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells", *J. Biol. Chem.*, Vol. 269, (1994), pp. 16896–16901.

[89] A. Stoica, B.S. Katzenellenbogen and M.B. Martin: "Activation of estrogen receptor-α by the heavy metal cadmium", *Mol. Endocrinol.,* Vol. 14, (2000), pp. 545–553

[90] W.B. Gill, G.F.B. Schumacher, M. Bibbo et al.: "Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities", *J. Urol.,* Vol. 122, (1979), pp. 36–39.

[91] S.G. Driscoll and S.H. Taylor: "Effects of prenatal maternal estrogen on the male urogenital system", *Obstet. Gynecol.,* Vol. 56, (1980), pp. 537–542.

[92] R.R. Newbold and J.A. McLachlan: "Transplacental hormonal carcinogenesis: diethylstilbestrol as an example", *Cellular and Molecular Mechanisms of Hormonal Carcinogenesis*, Wiley-Liss, New York, 1996.

[93] C. McKinnell, N. Atanassova, K. Williams et al.: "Suppression of androgen action

and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol", *J. Androl.,* Vol. 2, (2001), pp. 323–338.

[94] A.C. Belfroid, A. Van der Horst, A. D. Vethaak et al.: "Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands", *Sci. Total Environ.*, Vol. 225, (1999), pp. 101–108.

[95] A. Lagana, A. Bacaloni, G. Fago et al.: "Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry", *Rapid Commun. Mass Spectrom.,* Vol. 14, (2000), pp. 401–407.

[96] N. Atanassova, C. McKinnell, M. Walker et al.: "Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood", *Endocrinol.,* Vol. 140, (1999), pp. 5364–5373.

[97] R.M. Sharpe, A. Rivas, M. Walker et al.: "Effect of neonatal treatment of rats with potent or weak (environmental) oestrogens, or with a GnRH antagonist, on Leydig cell development and function through puberty into adulthood", *Int. J. Androl.,* Vol. 26, (2003), pp. 26–36.

[98] J.S. Fisher, K.J. Turner, D. Brown et al.: "Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood", *Environ. Health Perspect.*, Vol. 107, (1999), pp. 397–405.

[99] N. Atanassova, C. McKinnell, K. J. Turner et al.: "Comparative effects of neonatal exposure of male rats to potent weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility; evidence for stimulatory effects of low estrogen levels", *Endocrinol.,* Vol. 141, (2000), pp. 874–886.

[100] K. Williams, J.S. Fisher, K.J. Turner et al.: "Relationship between expression of sex steroid receptors and structure of the seminal vesicles after neonatal treatment of rats with potent or weak estrogens, tamoxifen, flutamide or a GnRH antagonist", *Environ. Health Perspect.,* Vol. 109, (2001), pp. 1227–1235.

[101] G.S. Prins and L. Birch: "The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen", *Endocrinol.,* Vol. 136, (1995), pp. 1303–1314.

[102] G.S. Prins, L. Birch, J.F. Couse et al.: "Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor alpha: studies with alpha ERCO and beta ERKO mice", *Cancer Res.,* Vol. 61, (2001), pp. 6089–6097.

[103] H. Adlercreutz and W. Mazur: "Phyto-estrogens and western diseases", *Ann. Med.*, Vol. 29, (1997), pp. 95–120.

[104] A.L. Murkies, G. Wilcox and S.R. Davis: "Phytoestrogens", *Clin. Endocrinol. Metab.,* Vol. 83, (1998), pp. 297–310.

[105] D.M. Tham, C.D. Gardner and W.L. Haskell: "Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence", *J. Clin. Endocrinol. Metab.,* Vol. 83, (1998), pp. 2223–2235.

[106] H.W. Bennetts, E.J. Underwood and F.L. Shier: "A specific breeding problem of sheep on subterranean clover pastures in Western Australia", *Aust. Vet. J.*, Vol. 22,

(1946), pp. 2–12.

[107] J. Kumi-Diaka and J. Townsend: "Effects of genistein isoflavone (4',5',7-trihydroxyisoflavone) and dexamethasone on functional characteristics of spermatozoa", *J. Med. Food,* Vol. 4, (2001), pp. 39–47.

[108] F. Paris, N. Servant, J.P. Gavedi et al.: "Antiandrogenic activities of phyto- and mycoestrogens: Natural endocrine disruptors?", *Horm. Res.,* Vol. 64, (2005), p. 25.

[109] R.A. Meronuck, K.H. Garren, C.M. Christensen et al.: "Effects on turkey poults and chicks of rations containing corn invaded by *Penicillium* and *Fusarium* species", *Am. J. Vet. Res.,* Vol. 31, (1970), pp. 551–555.

[110] K. Roine, E.L. Korpinen and K. Kallela: "Mycotoxicosis as a probable cause of infertility in dairy cows", *Nord. Vet. Med.,* Vol. 23, (1971), pp. 628–633.

[111] W. Powell-Jones, S. Raeford and G.W. Lucier: "Binding properties of zearalenone mycotoxins to hepatic estrogen receptors", *Mol. Pharmacol* ., Vol. 20, (1981), pp. 35–42.

[112] J. Buckley, E. Willingham, K. Agras et al.: "Embryonic exposure to the fungicide vinclozolin causes virilization of females and alteration of progesterone receptor expression in vivo: an experimental study in mice", *Environ. Health*, Vol. 5, (2006), pp. 4–10.

[113] M.D. Anway, M.A. Memon, M. Uzumcu et al.: "Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis", *J. Androl.*, (2006), ahead of print.

[114] M. Muramatsu and S. Inoue: "Estrogen receptors: how do they control reproductive and non-reproductive functions?", *Biochem. Res. Commun.,* Vol. 270, (2000), pp. 1–10.

[115] C.A. Blake, F.R. Boockfor, J.U. Nair-Menon et al.: "Effects of 4-tert-octylphenol given in drinking water for 4 months on the male reproductive system of Fischer 344 rats", *Reprod. Toxicol.,* Vol. 18, (2004), pp. 43–51.

[116] F.R. Boockfor and C.A. Blake: "Chronic administration of 4-tert-octylphenol to adult male rats causes shrinkage of the testes and male accessory sex organs, disrupts spermatogenesis, and increases the incidence of sperm deformities", *Biol. Reprod.,* Vol. 57, (1997), pp. 267–277.

[117] S. Khurana, S. Ranmal and N. Ben-Jonathan: "Exposure of newborn male rats and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression", *Endocrinol.,* Vol. 12, (2000), pp. 4512–4516.

[118] R. Steinmetz, N.G. Brown, D.L. Allen et al.: "The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo", *Endocrinol.,* Vol. 138, (1997), pp. 1780–1786.

[119] K.G. Nelson, Y. Sakai, B. Eitzman et al.: "Exposure to diethylstilbestrol during a critical developmental period of the mouse reproductive tract leads to persistent induction of two estrogen-regulated genes", *Cell. Growth Differ.,* Vol. 5, (1994), pp. 595–606.

[120] S. Li, K.A. Washburn, R. Moore et al.: "Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus", *Cancer Res.,* Vol. 57, (1997), pp. 4356–4359.

[121] W.W. Huang, Y. Yin, Q. Bi et al.: "Developmental diethylstilbestrol exposure alters genetic pathways of uterine cytodifferentiation", *Mol. Endocrinol.,* Vol. 19, (2005), pp. 669–682.

[122] D.E. Frigo, K. A. Vigh, A. P. Struckhoff et al.: "Xenobiotic-induced TNF-alpha expression and apoptosis through the p38 MAPK signaling pathway", *Toxicol. Lett.*, Vol. 155, (2005), pp. 227–238.

[123] K. McElreavey and L. Quintana-Murci: "Y chromosome haplogroups: A correlation with testicular dysgenesis syndrome?", *APMIS,* Vol. 111, (2003), pp. 106–114.

[124] T. Ogata, R. Yoshida, T. Hasegawa et al.: "Association of cryptorchidism with a specific haplotype of the estrogen receptor α gene: implication for the susceptibility to estrogenic environmental endocrine disruptors", *Horm. Res.,* Vol. 64, (2005), p. 34.

[125] E. Wolff and Ginglinger A: "Sur la transformation des Poulets males en intersexues par injection d'hormone femelle (folliculine) aux embryons", *Archs Anat. Histol. Embryol.,* Vol. 20, (1935), pp. 219–278.

[126] J.F. Couse and K.S. Korach: "Estrogen receptor null mice: what have we learned and where will they lead us?", *Endocr. Rev.,* Vol. 140, (1999), pp. 378–417.

[127] J. Takeyama, T. Suzuki, Inoue S. et al.: "Expression and cellular localization of estrogen receptors alpha and beta in the human fetus", *J. Clin. Endocrinol. Metab.,* Vol. 86, (2001), pp. 2258–2262.

[128] M. Nielsen, S. Bjornsdottir, P.E. Hoyer et al.: "Ontogeny of oestrogen receptor alpha in gonads and sex ducts of fetal and newborn mice", *J. Reprod. Fertil.,* Vol. 118, (2000), pp. 195–204.

[129] A. Ferlin and C. Foresta: "Insulin-like factor 3: a novel circulating hormone of testicular origin in humans", *Ann. N. Y. Acad. Sci.,* Vol. 1041, (2005), pp. 497–505.

[130] J.P. Bonde: "Occupational hazards to male fecundity", *Reprod. Med. Rev.*, Vol. 4, (1995), pp. 59–73.

[131] A.B. Wisniewski, S.L. Klein, Y. Lakshmanan et al.: "Exposure to genistein during gestation and lactation demasculinizes the reproductive system in rats", *J. Urol.*, Vol. 169, (2003), pp. 1582–1586.

[132] A.B. Wisniewski, A. Cernetich, J.P. Gearhart et al.: "Perinatal exposure to genistein alters reproductive development and aggressive behavior in male mice", *Physiol. Behav.,* Vol. 84, (2005), pp. 327–334.