



Review

The endocrine disruptors: a major medical challenge

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Summary

Endocrine disruptors (EDs), chemicals capable of disrupting the normal functioning of the endocrine system, may pose a growing threat to human and wildlife health. These compounds can modulate both the endocrine and immune systems resulting in alteration of homeostasis, reproduction, development and behavior. The hypothesis that chemicals in the environment have caused endocrine disruption is discussed along with important issues in the assessment of the risk of such disruption. Emphasis is put on the most significant pathological effects, namely impacts on the male reproductive tract, female gynecological system, human fertility, thyroid function and the central nervous system. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the last 40 years much attention has been focused on the potential for a wide range of xenobiotic chemicals to interact with and disrupt the endocrine systems of animal and human populations. Substantial evidence has surfaced on the hormone-like effects of environmental chemicals such as pesticides and industrial chemicals. The endocrine and reproductive effects of these chemicals are believed to be due to their ability to: (a) mimic the effect of endogenous hormones; (b) antagonize the effect of endogenous hormones; (c) disrupt the synthesis and metabolism of endogenous hormones; (d) disrupt the synthesis of hormone receptors. The discovery of hormone-like activity of these compounds occurred long after they were released into the environment.

The first human health effects were found in aviation crop-dusters handling DDT, who had reduced sperm counts (Singer, 1949). Also industrial workers at plants producing chlordecone (kepone) were reported to have lost their libido, became impotent and had low sperm

counts; subsequently experiments in laboratory animals demonstrated clearly the estrogenic activity of those pesticides (Guzelian, 1982). Another conclusive finding showed that man-made compounds used in the manufacture of plastics in laboratory materials were estrogenic because they fouled experiments studying natural estrogens (Soto et al., 1991). The chemicals responsible were nonylphenol and bisphenol-A. It is known that alkyphenol polyethoxylates are used in the synthesis of detergents and as antioxidants. They are not estrogenic; however, upon degradation during sewage treatment they may release estrogenic alkyphenols (Sonnenschein and Soto, 1998).

Nonoxynol, a surfactant, has been used as an intravaginal spermicide and also as a condom lubricant. This chemical, when administered to experimental animals, is metabolized to free nonylphenol (Knaak et al., 1966). Bisphenol-A was also found in dental sealants, thus leaching from the treated teeth into saliva, where it could be measured (Olea et al., 1996). Other xenoestrogens more recently identified include the plasticizers benzylbutylphthalate, dibutylphthalate, the antioxidant butylhydroxyanisole, the rubber additive *p*-phenylphenol and the disinfectant *o*-phenylphenol (Giam et al., 1978). Some compounds are cumulative. They have been found in sewage outlets in several rivers in the United Kingdom. Near these outlets were found feminized male fish (Purdom et al., 1994).

Estrogen mimics are just one class of endocrine disruptors (EDs). More recently, other compounds with

Abbreviations: APEs, alkyphenol polyethoxylates; DDE, dichlorodiphenyldichloroethene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; Eds, endocrine disruptors; PBDEs, polybrominated diphenyl ethers; PCB, polychlorinated biphenyl; PBDE, polybrominatedbiphenylether; PCDD, polybrominateddibenzodioxin; PCDF, polychlorodibenzofuran; TCDD, trichlorodibenzodioxin.

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anti-androgenic activity have been identified among environmental chemicals such as vinclozin, a fungicide, and dichlorodiphenyldichloroethene (DDE), a metabolite of the insecticide DDT. Moreover, a single compound may produce neurotoxic, estrogenic and anti-estrogenic effects. It has been suggested that EDs may be responsible for the decrease in the quantity and the quality of human sperm during the last five decades, as well as in the increased incidence of testicular cancer, prostate cancer and cryptorchidism in males, and breast cancer and endometriosis in females in the industrialized world (Colborn et al., 1993). In this review on the potential effects of Eds, emphasis is put on the human health effects, namely the relevant pathological aspects of the male reproductive tract, breast cancer and endometriosis in females, human fertility, thyroid function, CNS and neuroendocrine effects as well as the concentrations of EDs in human food and tissues and the relative potency of these chemicals in vitro as compared to oestradiol.

1.1. What are the endocrine disrupters?

EDs are defined as “an exogenous substance or a mixture, that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (European Commission, 1996). Concern over the possible consequences of exposure to xenobiotic compounds able to modulate the endocrine system has drawn the attention of international agencies, like the European Commission, the European Parliament, the US Environmental Protection Agency, the Organisation for Economic Cooperation and Development, the WHO International Programme on Chemical Safety, non-governmental organizations, and the chemical industry (OECD, 1989; CSTEE, 1999).

2. Mechanisms of endocrine disruption

The biological actions of hormones synthesized within an organism, such as estrogen, progesterone, testosterone and thyroxine, are mediated by high-affinity receptor proteins located within target cells. The interaction of a hormone with its receptor initiates a cascade of events that lead to the myriad of effects associated with the particular hormone. Some exogenous chemicals may bind to a receptor and mimic or block the actions of its natural hormone. Such compounds include some naturally occurring chemicals, such as coumestrol and genistein, pharmaceuticals such as diethylstilbestrol, 17 α -ethinyloestradiol and tamoxifen, and industrial chemicals such as DDT, bisphenol-A and nonylphenol (DFG and Eisenbrand, 1996; Schäfer et al., 1996). Compounds that have been shown to alter estrogen

biosynthesis are cyanoketone, ketoconazole and the fungicide fenarimol (Hirsch et al., 1987). Methoxychlor, chlordecone (kepone), DDT, some PCBs and alkylphenols can disrupt estrogen receptor function (Mueller and Kim, 1978; White et al., 1994). Metabolites of the fungicide vinclozin and the DDT metabolite *p,p'*-DDE have been found to bind to the androgen receptor and block testosterone-induced cellular responses in vitro. Ligand binding to the estrogen and progesterone receptors may be inhibited by *o,p'*-DDT and chlordecone (Laws et al., 1995). Nonylphenol and other alkylphenol polyethoxylates (APEs) inhibit binding to estrogen receptors (CMA, 1993).

In mammals, the steroidal sex hormones and their receptors have a regulatory role in developmental processes such as sex determination and differentiation. The development of the conceptus into a male phenotype is regulated by testicular hormones including testosterone. The androgen receptor antagonists *p,p'*-DDE and vinclozin may demasculinize male rat pups following exposure of the pregnant dam (Kelce et al., 1997). In birds, differentiation into a female phenotype is regulated by estrogen. Injection of an estrogen synthesis (aromatase) inhibitor into the fertilized egg may result in phenotypically sex-reversed females with bilateral testicles, sperm production and male copulatory behaviour (Elbrecht and Smith, 1992). Feminized male birds with an ovotestis and/or reduced male copulatory behaviour have been induced by *o,p'*-DDT and 17 α -ethinyloestradiol (Berg et al., 1998).

In lower vertebrates and non-vertebrates, the mechanisms of sexual differentiation are less well characterized but may be more flexible than in mammals and birds. They are influenced by environmental factors such as temperature and xenogenous substances with hormonal activity (Campbell and Hutchinson, 1998). Experimentally disturbed sexual differentiation has also been reported in reptiles, amphibia, fish and non-vertebrates such as gastropods. There is also evidence that malformed sex organs occur in wild populations of birds, reptiles, fish and marine gastropods. A comprehensive review of this multidisciplinary research field is extensively dealt with in the CSTEE Report (CSTEE, 1999). The mechanisms of pollutant-induced reproductive toxicity observed in wild mammalian species remain unclear but could involve endocrine disruption.

3. Endocrine disrupters and human health

The available evidence for the human health effects of EDs on the male reproductive tract, on certain types of male and female cancers, on fertility, on the thyroid, and on the central nervous system, as well as exposure considerations, are briefly summarized.

3.1. Decreased sperm quality

Although controversy persists about the allegation that human sperm production has been declining worldwide during the past 50 years, a hypothesis has been proposed that in utero exposure to environmental estrogens might be responsible (Sharp and Skakkebaek, 1993). A meta-analysis of 61 studies that included about 15,000 men found a substantial decrease in sperm concentration from $113 \times 10^6/\text{ml}$ in 1938 to $66 \times 10^6/\text{ml}$ in 1990, and in semen volume from $3.40 \times 10^{-3}/\text{l}$ in 1938 to $2.75 \times 10^{-3}/\text{l}$ in 1990 (Carlsen et al., 1992). This study can be questioned since it suffers from various sources of bias and confounding. Geographical and chronological studies showed a big variability either in the quality or in the quantity of sperm (Jensen et al., 1995; Mees et al., 1997). There are several additional studies of sperm quality that have a relative good design (Fish et al., 1996; Paulsen et al., 1996). These studies observed an unchanged seminal volume or even a slight increase. Additional carefully designed epidemiological studies are required to substantiate and clarify the cause of any reduction of sperm counts. A reduced sperm count is not necessarily associated with infertility (Seibert, 1997).

3.2. Cryptorchidism and hypospadias

An increase in the prevalence of cryptorchidism was reported in the previously mentioned study (Jensen et al., 1995). Very few studies have examined temporal changes in prevalence. Therefore, additional epidemiological investigations are needed. The same studies have also reported a temporal increase in the prevalence of hypospadias, primarily in England and Wales, Hungary, Sweden, Norway, Denmark, Finland, Spain, New Zealand, Australia and the Czech Republic (Jensen et al., 1995). A higher prevalence for whites than in blacks in the USA was not confirmed (Berkowitz et al., 1995).

3.3. Testicular cancer

The incidence of testicular cancer in men has increased significantly during the last decades (Forman and Moller, 1994). The tumors are primarily germ cell in origin. The incidence of cancer in men under 50 years of age has increased approximately 2–4% per annum since the 1960s in Great Britain, the Nordic and Baltic countries, Australia, New Zealand and the United States (Toppari et al., 1995). Testicular cancer is the most common malignancy among men age 25–34 years in Denmark, while in Finland the incidence is much lower (Adami et al., 1994). It has not yet been possible to conclude whether the apparent increase in testicular cancer in many countries is due to hormonally substances, to changed life-style conditions or to other causes.

3.4. Prostate cancer

Cancer of the prostate is the second leading cause of cancer deaths in males in the USA. Death due to prostate cancer has increased by 17% over the past 30 years despite improved diagnosis. There are racial differences in susceptibility, the prevalence being rare in Orientals, 20–30 times higher in Caucasians, and even higher in Afro-American males (Crisp et al., 1998, pp. 11–56). A meta-analysis found a positive association between prostate cancer and farming occupation (Keller-Byrne et al., 1997). Little is known about the causes of prostatic cancer but age, genetics, endocrine status, diet and environmental risk factors have been proposed.

3.5. Breast cancer

Cancer of the breast is the most frequent tumor in women in the world. The relative frequency varies five-fold between countries; the highest incidences are found in western Europe and in North America (Coleman et al., 1993). There is a two-fold difference between the highest and lowest incidence rates in Europe, as is found in Switzerland compared with Spain. There has been a steady increase in breast cancer incidence rates over the last decades everywhere in Europe. Estimated mean changes per 5-year period in age-specific rates (30–74 years) over the period 1973–87 are in the order of 5–25%. Several factors have been identified as potentially responsible for increasing the risk, but their mechanism of action is unclear. Reproductive history obviously plays a role since early menarche, late first pregnancy, low parity and late menopause are all associated with an increased risk of breast cancer. Estrogenic influences may be important since oophorectomy has a protective effect on breast cancer development (Coleman et al., 1993).

The increased risk of breast cancer has been suggested to be related to exposure to estrogenic chemicals. Several case-control studies published in the last two decades have raised the issue that women exposed to organochlorine chemicals such as DDT and certain PCB congeners may have higher incidences of breast cancer than non-exposed women (Dewailly et al., 1994). For example, plasma concentration of dieldrin was positively associated with an increase risk of breast cancer in a study including 268 cases from a cohort of 7712 women; in the same study there was no association with total PCB, total DDT or HCB in plasma (Høyer et al., 1998). However, other studies have failed to find associations between organochlorine exposure and breast cancer (Hunter et al., 1997). A systematic review of the epidemiologic findings regarding the association between organochlorine and breast cancer found these to be inconclusive (Ahlborg et al., 1995). Available

studies support a possible weak association between diethylstilbestrol (DES) exposure during pregnancy and later development of breast cancer (Colton and Greenberg, 1998).

Future experimental “hot-spots” observations, in areas linking ecotoxicological and epidemiological studies, may help to identify whether there is any association between organochlorine exposure and breast cancer. There is no evidence from experimental studies that DDT, DDE or PCB produce increased incidences of mammary tumors in rodents. However, administration of DES, genistein and *o,p'*-DDT has resulted in enhanced epithelial cell proliferation and differentiation of abdominal mammary glands in female rats (Brown and Lamartinière, 1995). In another bioassay, TCDD, which may act as an anti-estrogen, reduced the number of spontaneous tumors in the mammary gland in a 2-year bioassay (Kociba et al., 1978).

3.6. Endometriosis

Endometriosis is characterized by aberrant growth of endometrial cells outside the uterus and ensuing dysmenorrhea due to sloughing of the estrogen-induced proliferation tissue and the internal bleeding that follows. Prevalence estimates range widely with an average of 10% and it affects 5 million women in the USA (Holloway, 1994). The etiology of this disease is unknown, but exposure to estrogen-like compounds has been hypothesized and immune mechanisms may be involved in the disease process (Hill, 1992). Some studies reported increased risks of endometriosis in women formerly taking oral contraceptives, whereas there was a decreased risk in women currently taking such medication (Vessey et al., 1993). Also there is evidence of an increase frequency of endometriosis in female offspring exposed in utero to diethylstilbestrol (Berger and Alper, 1986). An association between endometriosis and high levels of PCB in plasma has also been reported (Gerhard and Runnebaum, 1992). Contradictory results have been reported in a small study looking at the association between endometriosis and serum levels of PCDD, PCDF or PCB. In this study the disease status was determined by laparoscopy (Boyd et al., 1995).

In an investigation in rhesus monkeys, a dose response between dioxin exposure via the diet and the incidence and severity of endometriosis was observed (Rier et al., 1993). In another study, dietary PCB exposure did not alter endometrial lesions (Arnold et al., 1996). In more recent studies with rats and mice, TCDD was found to promote endometriosis in both species (Cummings et al., 1996). More epidemiological studies addressing possible links between exposure to hormonally active xenobiotics and endometriosis are needed.

3.7. Effects on fertility

Infertility may be defined as a couple's failure to conceive after a period of 1 year of unprotected intercourse and may affect between 10 and 15% of couples. It is estimated that 50% of all fertility problems may be related to male reproductive function (Swerdloff, 1985). Although one study found three times more genital malformations in men with in utero exposure to DES than controls, there was no significant difference in fertility (Wilcox et al., 1995). Increases in blood levels of *o,p'*-DDT, DDD, DDE, lindane, tetra- and pentachlorobiphenyls have been found more frequently in individuals with a lower sperm count (Pines et al., 1987). However, the PCB exposures in individuals exposed occupationally may be quite different from the pattern following dietary exposure.

Conflicting results have come from studies examining fertility and pregnancy outcomes of women exposed in utero to DES. Whereas some studies report no differences in fertility rates (Barnes et al., 1980), another study reported significant differences (Bibbo and Gill, 1977). However, it is well established that exposure in utero leads to greater incidence of unfavorable pregnancy outcomes such as miscarriage, ectopic pregnancy, stillbirth and premature birth (Barnes et al., 1980). It is known that daily oral intakes of ethinylestradiol plus varying amounts of progesterone are required to cause infertility by oral contraceptives (Isselbacher et al., 1994).

3.8. Effects on sex ratio

Male to female sex ratio has been used as an indicator of effects on human reproduction. After the Seveso accident with exposure to dioxin, there was an excess of females born in the period April 1977 to December 1984 (Mocarelli et al., 1996). This ratio declined in the years from 1985 to 1994 and was thereafter no longer significant. There have also been reports of declining proportion of male newborns during the last decades from Denmark (Moller, 1996), The Netherlands (Van der Pal-de Bruin et al., 1997), England and Wales (Dickinson and Parker, 1996) as well as from Canada (Allan et al., 1997). These declines in the sex ratio remain unexplained, but have been speculated to be related to exposure to xeno-estrogenic compounds.

3.9. Effects on the thyroid and neurobehaviour

Thyroid hormones play a key role in the maintenance of body homeostasis. Altered status may lead to changes in basal metabolism rate, lipid metabolism, as well as cardiovascular, gastrointestinal and muscle function. Thyroid hormones are important during growth and development, especially in the maturation of the brain.

A number of environmental agents can alter thyroid hormone levels in humans and animals (Capen, 1992; Crisp et al., 1998). Hypothyroidism in rodents has been observed after exposure to PCB, TCDD, and chlorinated pesticides (Crisp et al., 1998). There are several studies which show that PCBs produce a wide spectrum of neurochemical and neuroendocrine effects in animals (Tilson and Kodavanti, 1997). Higher PCDD, PCDF and PCB levels in human milk have been found to correlate with lower plasma levels of maternal thyroid hormones and with higher plasma levels of thyroid stimulating hormones in infants shortly after birth (Koopman-Esseboom et al., 1994). In addition, hydroxylated PCBs have been found to be potent competitive inhibitors of thyroxine binding to the human thyroid hormone transport protein, transthyretin (Lans et al., 1993).

3.10. Neuroendocrine effects

Children born to women exposed to high levels of PCB/PCDF via consumption of contaminated fish oil or rice oil have been reported to show delayed mental development with lower IQ scores, cognitive dysfunction, poorer visual recognition memory and behavioural difficulties (Hsu et al., 1985). A Dutch study observed a significant delay in psychomotor development in children pre- and postnatally exposed to lower environmental levels of PCBs, PCDDs and PCDFs (Koopman-Esseboom et al., 1996). In a US study, developmental neurotoxicity involving cognitive and neurobehavioral disturbances have been reported following perinatal exposure to PCBs from environmental pollutants (Jacobson and Jacobson, 1996). Enhanced levels of these environmental contaminants in breast milk have been related to reduced neonatal neurological function (Huisman et al., 1995). Polybrominated diphenyl ethers (PBDEs) have also been identified in human plasma and milk samples. Neonatal exposure to some PBDE congeners may cause disturbed spontaneous behavior similar described for PCBs (Eriksson et al., 1997). However, at present it is not known whether the reported developmental neurotoxicities related to PCB/PCDF/PCDD/PBDE exposures are caused by endocrine mechanisms.

3.11. Food exposure considerations

Food is likely to be the most important route of exposure to EDs for humans. There have been a number of investigations on the concentrations of putative and known EDs in human food and tissues and the relative potency of these chemicals in vitro as compared to 17 β -estradiol (Gaido et al., 1997; Zava and Duwe, 1997). For assessing the relative risk of EDs, human exposure to these compounds expressed as their concentration in serum was related to the estrogenic activity determined in in vitro tests as the effective concentration

of the compounds showing 505 or 100% of the maximum activity of a fixed amount of 17 β -estradiol. Estrogenic activities are estimated from experiments using test systems such as competitive binding to estrogen receptors in human breast cancer MCF-7 cells, proliferation of MCF-7 cells (E-SCREEN), or expression of a reporter gene in the yeast estrogen system (YES). The results show that the relative potencies of *o,p'*-DDE, PCBs, nonylphenol, bisphenol A and dieldrin in vitro systems are several order of magnitude lower than that of 17 β -oestradiol (Sonnenschein et al., 1995; Soto et al., 1995).

The role of natural estrogens in the diet should also be considered. The intake of phytoestrogens from food varies widely among different populations, depending on their dietary habits (Cassidy, 1988). The phytoestrogen genistein, for example, with both a higher estrogen activity and higher serum concentrations than other putative EDs, has a relative potency which may even exceed that of estradiol in the case of diets rich in soy. Soy protein (60 g/per day), containing estrogenic isoflavones, has been shown to prolong the follicular phase of the menstrual cycle in women (Cassidy, 1988). Naturally occurring dietary estrogens have activities ranging from 1/500 to 1/1000 of 17 β -estradiol (Safe, 1995).

The estimated daily human intake of estrogenic and anti-estrogenic equivalents, based on in vitro potencies relative to 17 β -estradiol, has indicated that a woman taking a birth control pill ingests about 16,675 μ g equivalents per day, postmenopausal estrogen therapy amounts to 3350 μ g, and ingestion of estrogen flavonoids in food represents 102 μ g, whereas daily ingestion of environmental organochlorine estrogenic equivalents was estimated to be 0.0000025 μ g.

However, it should be realized that these in vitro potency comparisons could be misleading. For example, they do not take into account whether binding to sex hormone binding globulin in plasma may be relatively greater for the endogenous hormones than for exogenous EDs. Similarly, they do not take account of the rates of absorption and metabolism of exogenous EDs in vivo. Comparisons measuring only binding to estrogen receptors do not take account of differences between endogenous hormones and exogenous EDs in their ability to activate downstream events after receptor binding that ultimately lead to changes in gene expression.

It is evident that at present the dose–response or dose–effect relationship for most of the compounds to be considered is not well elucidated in man. Furthermore, for some hormonally active compounds the most sensitive target may not yet have been identified. All these factors, together with poor availability of exposure data, currently impose limitations on our ability to carry out risk assessments on EDs in humans.

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