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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Endocrine disrupters as environmental signallers: an introduction

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Abstract: After an historical overview of the endocrine damage to wildlife caused by environmental xenobiotics (to be distinguished from natural phyto-oestrogens), the most important groups of environmental pollutants endowed with endocrine activity are reviewed showing that the reproductive system is not the only apparatus affected although it is the most common target of the xeno-oestrogens. The mechanisms of action of putative endocrine disrupters are then discussed. In this hormonal context, the relationships between the latter compounds and breast cancer are discussed. The analytical approaches to identify environmental pollutants with oestrogen activity, in particular oestrogens, are also briefly reviewed. Finally, difficulties in correlating xenobiotic activity to reduced sperm counts, breast cancer and endocrine changes are highlighted.

INTRODUCTION

Some 30 years after Rachel Carson's insightful predictions [1], there is growing alarm over the potential adverse effects of environmental contaminants, particularly those that involve the endocrine/reproductive functions. In the late 1970s, male herring gull (*Larus argentatus*) embryos and chicks in the Lake Ontario area were noted to have oviducts and ovary-like gonads, and subsequent research revealed that pesticides such as dicofol, kelthane and methoxychlor were producing the same effects in the California gulls, western gull and kestrels (*Falco tinnunculus*) [2]. More recently, female-female couple formation has been described among herring gulls that have been contaminated with DDT, DDE and PCBs [3] (Table 1). Female white suckers (*Catostomus commersoni*—a freshwater fish found in North America) that are exposed to bleached kraft pulp milk effluent present significantly smaller ovaries than unexposed controls [6], and similar effects have been observed in other fish (e.g., *Colisa lalia*) exposed to the pesticide carbofuran. A study of juvenile adult alligators in Lake Apopka in Florida revealed alarming changes that are believed to be linked to the DDT-like pesticide, dicofol, which had polluted the lake during the 1980s. The animals presented extremely low levels of testosterone and abnormal testicular development. Moreover, their penises were roughly half the size of those of normal animals, and signs of feminisation were noted. Female alligators displayed substantially increased blood 17β-oestradiol levels and ovarian abnormalities [7–13]. Other investigators have reported hermaphrodite fish populations in the discharge pools of water-treatment plants [14].

A variety of other types of endocrine effects in wildlife have been tentatively attributed to environmental pollutants [15], and the planetary decline in certain species (e.g., certain amphibians) is suspected of being caused in part by reproductive difficulties caused by these chemicals [16,17]. Although most of the anomalies that have been reported involve the reproductive system, changes in

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other organs have also been observed. In the Great Lakes of North America, for example, all salmon over two years old have grossly enlarged thyroids, with follicles sometimes verging on rupture [18].

The so-called endocrine disrupters are also believed to have played a role in the increasing incidence of a number of anomalies in Man (e.g., testicular, prostate and breast cancer, cryptorchidism, developmental anomalies of the foetal reproductive organs, declining sperm counts). The problem here is that the effects of intrauterine exposure to these chemicals may not become apparent in humans for the first 10–15 years of life, i.e., until puberty occurs. But since the cellular make-up, hormonal control and embryonic development of fish, reptiles, birds and mammals (including humans) are roughly equivalent, the reactions of these organisms to pesticides and other contaminants should also be comparable. Moreover, the consequences of such exposure is much more evident in wild species, in part because of their shorter gestation periods and life cycles, but also because their level of exposure is generally greater than that of humans. The reactions of these animals can thus serve as an index of the risk to Man.

Table 1. Compounds identified as xeno-oestrogens (from Longnecker *et al.* [4] and Safe [5], integrated and modified [10])

Pesticide organochlorine compounds	
Class	DDT: dichlorodiphenyltrichloroethane
Dichlorodiphenylethanes	(*o*,*p*′-DDT: *o*,*p*′-dichlorodiphenyltrichloroethane)
	(*p*,*p*′-DDT: *p*,*p*′-dichlorodiphenyltrichloroethane)
	(*p*,*p*′-DDE: *p*,*p*′-dichlorodiphenyldichloroethane)
Cyclodienes	Endosulfan
	Dieldrin
Toxaphenes	Toxaphene
Other insecticides	Chlordecone
	p,*p*′ Methoxychlor
Non pesticides organobromine and organochlorine compounds	
Polybrominated biphenyls (PBBs)	
Polychlorinated biphenyls (PCBs)	
Polychlorinated dibenzofurans [TCDF: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin(dioxin) and OCDD: octachlorinated dibenzodioxin]	
Miscellaneous	
Bisphenol A	
Alkylphenols poliethoxylated (e.g. 4-nonylphenol, 4-octylphenol)	
Phtalates (benzyl, butyl and d-n-butylphtalate)	
Vinclozolin	
Dithiocarbamates	

In recent years the media has strongly emphasised the potential role of certain environmental pollutants in increasing the incidence of breast cancer [19–22], diminishing sperm counts and male reproductive capacity and compromising the neural development of infants and children. These are highly emotional issues, but their complexity must not be underestimated. In-depth examination of current thinking on some of these alarming phenomena can be found in pp. 1685–1701 (Male infertility), and pp. 1713–1723 (Breast cancer).

While it seems clear that a number of soil and ground-water pollutants that are absorbed via the food chain [23,24] (fish, meat, milk—including breast milk—and other dairy products [4]) are indeed capable of causing endocrine changes, particularly when exposure is prolonged and/or occurs during gestation, the issue is far from being resolved. A cause-effect link in these cases must be based on clear and unequivocal evidence from large-scale epidemiological studies of the case-control type, and exposure levels that pose a threat must be identified in long-term studies of animals treated on a daily basis with different doses. Unfortunately, as Cooper & Kavlock [13] have pointed out, exposure conditions created

in the laboratory are often quite different from those found in the wild. The latter situation is generally characterised by persistent, long-term exposure to minimal levels, which are almost always lower than those used in research settings.

To further complicate matters, the endocrine effects produced by the pollutants in question often have to be distinguished from those that might be produced by naturally occurring dietary compounds such as the phyto-oestrogens. For example, infertility has been documented in Australian sheep that grazed on red clover, which is a natural source of isoflavonoid oestrogens [25,26] and in captive cheetahs fed diets based on soy flour, which is rich in oestrogenic flavones [27]. In rats, certain phyto-oestrogens (e.g. isoflavonoids) and lignans compete with 17β-oestradiol for the type-II oestrogen binding site, which is also known as the 'bioflavonoid' receptor [28]. Phyto-oestrogens can alter the metabolism of sex hormones, and they can also influence the growth of certain tumours via their effects on blood levels of steroid hormone binding globulin (SHBG) and their direct inhibition of the growth and proliferation of hormone-dependent cancer cells [28–30]. High fibre foods containing phyto-oestrogens and lignans are, in fact, believed to exert protective effects against breast and prostate cancer respectively, but the actual potency of the phyto-oestrogens in humans is still unclear**.** The many questions surrounding the issue of naturally occurring dietary oestrogens are discussed in pp. 1759–1776 ('Naturally occurring oestrogens in food'), pp. 1777–1782 ('Dietary phyto-oestrogens and cancer') and pp. 1853–1860 ('Clover phyto-oestrogens').

THE MOST IMPORTANT ENDOCRINE DISRUPTERS

Pesticides and industrial chemicals

While numerous xenobiotics can be considered potential endocrine disrupters (see [13,15,30]), attention has been focused, for the most part, on persistent environmental pollutants with weak oestrogenic or anti-androgenic properties (see Tables 1 and 2).

Table 2. Examples of endocrine-disrupting environmental contaminants (modified from L.J. Guillette & E.A. Guillette [10])

Compound	Effects
Pesticides	
o,*p*'-DDT	oestrogenic: induces uterine growth in ovariectomized rodents; feminization in birds; vitellogenin (yolk protein) synthesis in male turtles and frogs.
p,*p*'-DDE	anti-androgenic: *in vivo* male (rodent) development studies; positive androgen receptor transfected yeast binding studies: demasculinization of male alligator neonates; induction of polyovular ovarian follicles in juvenile alligators
Endosulfan	oestrogenic: induction of proliferation in human breast oestrogen sensitive MCF7 cells
Toxaphene	oestrogenic: induction of proliferation in human breast oestrogen sensitive MCF 7 cells
Dieldrin	oestrogenic: induction of proliferation in human breast oestrogen sensitive MCF 7 cells
Atrazine	increased testosterone degradation in male rats; disrupt the control of GnRh
Aldicarb	depression in plasma thyroxine and somatotropin concentration in rodents
Vinclozolin	anti-androgenic metabolites: *in vivo* alters sex differentiation in male (rodent) by inhibition of androgen receptor-mediated gene activator; yeast androgen binding studies
Industrial chemicals	
Bisphenol-A, Alkylphenols poliethoxylated	oestrogenic: induction of proliferation in human breast oestrogen sensitive MCF7 cells; uterine growth in prepubertal rats; binding fish oestrogen receptor; vitellogenin (yolk protein) synthesis in male fish; reduced testicular size and sperm production in rats
Phtalates	oestrogenic: positive oestrogen receptor transfected yeast binding studies; reduced testicular size and sperm production in rats
Polychlorinated biphenyl	oestrogenic: induction of sex reversal in turtle embryos/altered steroid metabolism in rats.

Pesticide organochlorine compounds (POCs)

These include the pesticide organochlorine compounds 2,2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane (DDT), and the stable breakdown product of the latter, 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (*p,p'* DDE)] and their analogs, benzene hexachlorides, cyclodienes and toxaphenes (Table 1 and fig. 1) POCs have been found in fish and other forms of wildlife, as well as in human tissues, blood and breast milk.

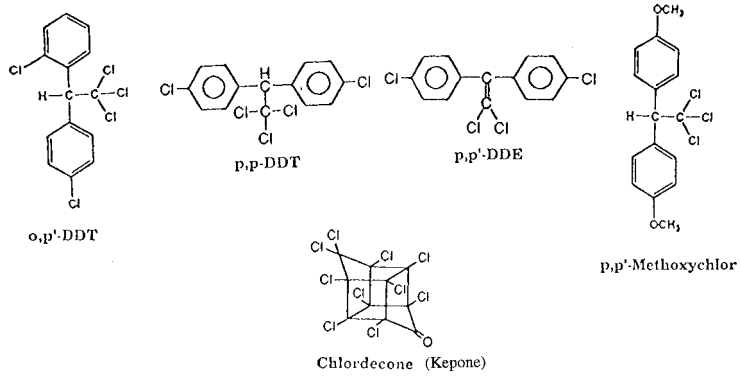


Fig. 1 Pesticide organochlorine compounds

One of the best known xeno-oestrogens is *o,p'* DDT [13,30], which is the most important oestrogenic contaminant of DDT. It binds and activates oestrogen receptors stimulating the expression of other receptors for progesterone and specific enzymes and the synthesis of uterine DNA [31]. *p,p'*-DDE is the DDT metabolite that persists longest *in vivo* [34], with a half life of over 65 years. This compound represents 50–80% of the total DDT-derivative residues found in human breast milk, and it acts primarily as an androgen antagonist [34]. Neither *o,p'* DDT or its metabolite are found years after exposure.

The DDT metabolite [2,2-bis-(*p*-hydroxyphenyl) 1,1,1-trichlorethane)] binds receptors for oestrogens, progesterone and androgens [32], and it has been shown to alter both the development and function of reproductive organs in rats [33]. Lindane is a mixture of isomers, one of which, b-HIH, is stable and weakly oestrogenic, although it has negligible insecticide activity. It is an atypical oestrogen in that it does not compete with oestradiol at a receptor level [31].

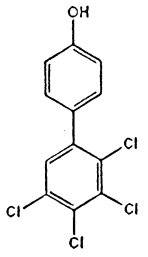
Chlordecone (kepone) [30,35], dieldrin [30,35,36,37], aldrin [35], endosulfan [36], toxaphene [36,37] and linuron [38] all display oestrogenic and anti-androgenic properties [13,39]. Chlordecone and *o,p'* DDD are similar in their affinities for oestrogen and progestin receptors [13]. Chlordecone is also believed to have an impact on human reproductive function: a higher than normal incidence of testicular changes has, in fact, been reported among workers exposed to this pesticide for long periods of time [40].

Chlorophenolic pesticides, chlorophenoxyl acids, and organochlorines can modify circulating levels of thyroxin (T_4) and alter thyroid function. It seems that they enhance hepatic metabolism of T_4, and displacement of the T_4-binding globulin in the blood and affect the cytochrome P450 enzyme system leading to altered hepatic metabolism of androgens [39].

Nonpesticide organobromine and organochlorine compounds

A number of oestrogenic polychlorinated biphenyl ((PCB) and PCB-related substances have been found in fish and other forms of wildlife, as well as in human tissues, blood and breast milk characterised by long persistence. Certain PCB metabolites found in plasma are actually oestrogen antagonists [41,42,43].

Certain PCBs are known to alter thyroid function. Those most commonly found in human tissues contain chlorine in 6 or 7 [4]. Exposure to these compounds can lead to alterations in higher functions in the adult, but it is important to recall that the thyroid can also influence development of the gonads [13].

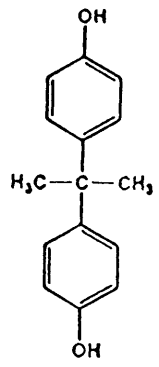


Hydroxy-PCB

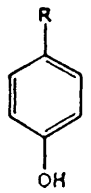
Miscellaneous

Ethylene-2-methylsulfonate interferes with the synthesis of testosterone [41]. Atrazine causes false pregnancies, prolongs dioestrus [44] and produces breast tumours [45]. The constant state of oestrus may be responsible for this latter effect. In any case, atrazine alters hypothalamic control of hypophyseal-ovarian function [13,44]. Certain formamide acaricides such as chlordimenformon seem to cause similar interference by blocking the α_2-adrenergic receptors and consequently abolishing noradrenergic control of gonadotropin-releasing hormone (GnRH) [46].

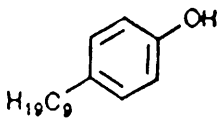
Alkylphenolic chemicals (e.g. 4-octylphenol, 4-nonylphenol, bisphenol A) composed of an alkyl chain containing at least three carbons and a backbone chain based on C-C (rather than C-O) bonds may also display oestrogenic activity [47,48,49,50,51]. These compounds, which are biodegradation products of nonionic surfactants (alkyl phenol polyethoxylates, APEOS) and laboratory plasticware (e.g. 4-nonylphenol, bisphenol A) [30,49,52], are suspected of altering reproduction in fish [14,27,52–55]. The metabolite of one of these compounds, *p*-nonylphenol (like methoxychlor metabolites) binds oestrogen, progestin and androgen receptors [55] (Fig. 2). It is interesting to note that specimens collected from certain drinking-water sources have been found to contain 20 or more alkylphenol-related compounds [30].



Bisphenol-A



p-Alkylphenols



4-Nonylphenol

Fig. 2. Bisphenol-A and alkylphenol derivatives

The fungicide vinclozolin [3-3,5-dichlorophenyl)-5-methyl-5-vinyl-oxazolinidine-2,4-dione)] is an anti-androgenic whose metabolites [34,56] act at the receptor level (Fig. 3). It reportedly alters sexual differentiation in male rats [57].

Dithiocarbamates and substances like carbon disulfide that form dithiocarbamates in cells can cause changes in ovulation and pregnancy. These effects are probably related to the inhibition of noradrenaline synthesis that they cause in rodents, birds and humans, a phenomenon that might cause disturbances at the central level in hypothalamic regulation of the hypothalamo-pituitary-gonad axis [13,58].

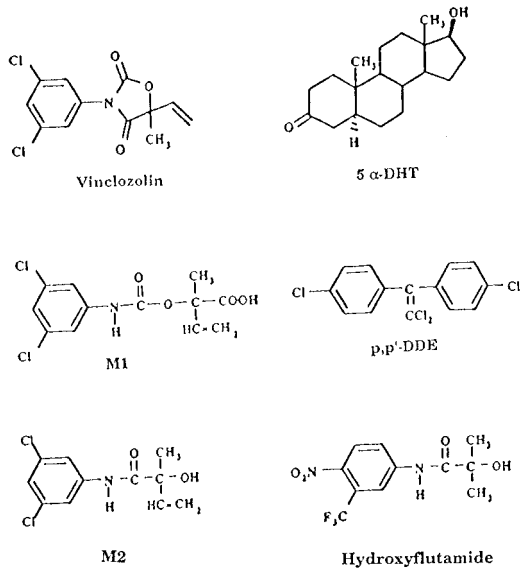


Fig. 3. Structural formulas of vinclozolin, vinclozolin metabolites M1 and M2, dihydrotestosterone (DHT), DDE, and hydroxyflutamide (an aniline-substituted derivative of flutamide with potent antiandrogenic properties that has been used in the treatment of prostatic carcinoma [34]).

2,3,7,8 Tetrachlorodibenzo-*p*-dioxin (TCDD) (fig. 4) does not appear to produce any endocrine effects that are directly mediated by oestrogen receptors [59]. Therefore, anomalies observed in animals exposed to this compound such as testicular atrophy [60], reduced biosynthesis and circulating levels of testosterone (also observed in humans in association with increased gonadotropin concentrations) [61], ovarian atrophy [62], and overall negative actions on the reproductive system [63,64,65] seem to be specifically mediated by the arylhydrocarbon (*Ah*) receptor type, i.e., a paradigm of the nongenomic receptor. Dioxin is not then a true anti-oestrogen although it displays an anti-oestrogenic profile; neither can it considered an oestrogen, an androgen or an anti-androgen [5]. It is also possible, however, that the endocrine effects attributed to TCDD might actually be caused by the presence in foods of indolecarbinol (found in broccoli and other foods), which specifically binds *Ah* receptors [66] (see fig. 4).

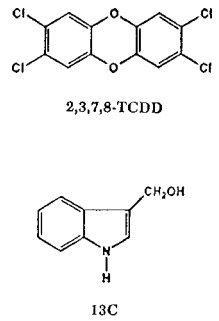


Fig. 4. 2,3,7,8 Tetrachlorodibenzo-*p*-dioxin (TCDD) and a compound that bind to the AhR. [5]

Phyto-oestrogens

The phyto-oestrogens include: flavonoids (flavanones, flavones, flavonols, hydroxy-chalcones, isoflavonoids such as daidzein, genistein, equol, and β-sitosterol), isoflavans, coumestans, lignans, and myco-oestrogens such as zearalenone, a resorcilic acid lactone, and its synthetic derivative zearalanol (fig. 5) [28–30].

The oestrogenic isoflavone content of most soy proteins is only 0.1–0.2% [67], most of which is represented by daidzein and genistein. The latter are transformed by the bacterial flora of the intestine into equol (Fig. 5), which is more potent than either genistein or daidzein, but its capacity for inducing corneification of the vaginal epithelium in post-menopausal women is still quite limited (19% as opposed to 8% among controls) [68].

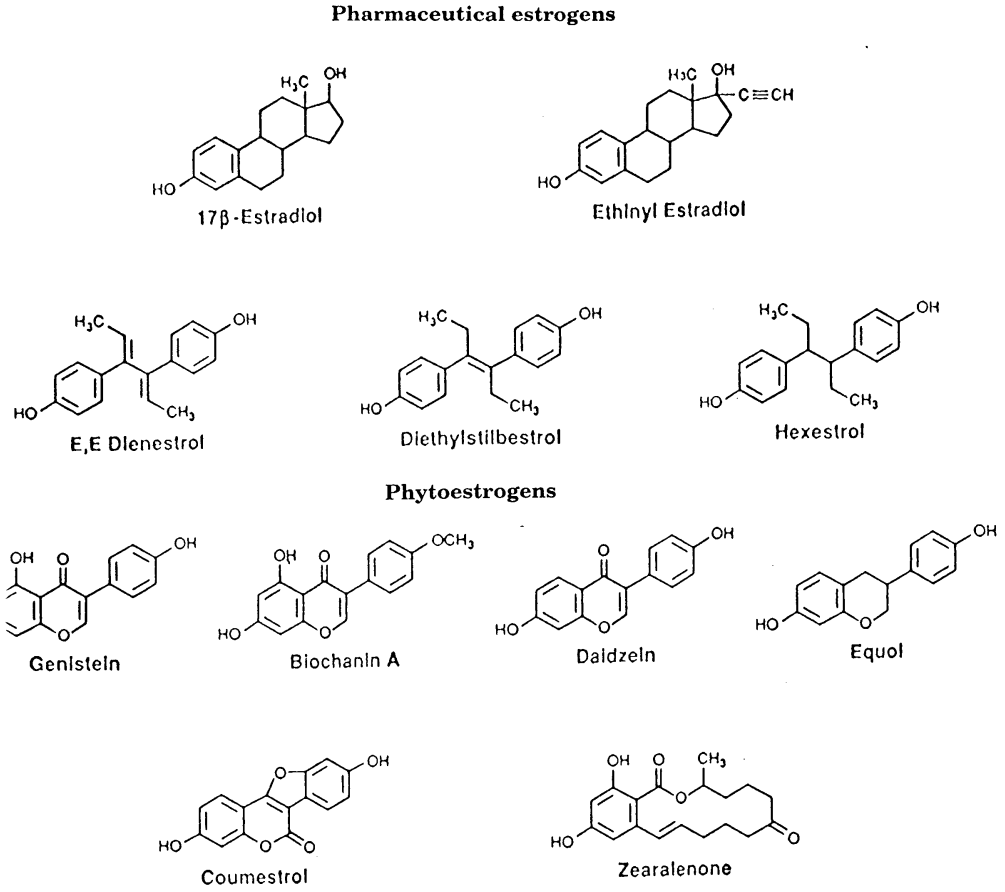


Fig. 5 Chemical structures of a variety of pharmaceutical and dietary oestrogens [Modified from Korach *et al.* (30)]

β-sitosterol [69] can cause infertility in livestock (e.g., sheep) that graze on plants rich in phyto-oestrogens [70]. β-sitosterol is capable of inducing vitellogenin (see below) in male fish, and it provokes an oestrogenic response in MCF-7 and T47D cells [71]. However, it can also decrease gonadal production in fish by interfering with cholesterol synthesis or inhibiting cytochrome P450 [34].

Plants also contain anti-androgens such as permixon, which is found in a Florida palm known as *Seronoa repens B* and used to prevent prostatic hypertrophy [33].

It should also be pointed out that a glucosinolate complex referred to as I3C has been detected in cruciferae such as broccoli, cauliflowers, cabbages and Brussels sprouts. I3C releases a free indolic fraction in the intestinal tract, which antagonizes oestrogen activity in endometrial carcinoma and competes with dioxin (TCDD) for binding to specific *Ah* receptors [5] (Fig. 4).

Structural characteristics of the xeno-oestrogens

Although the endocrine disruptors can also have effects on glands and neuroendocrine systems that are unrelated to reproduction, most of the interest these compounds have aroused has undoubtedly been focused on their xeno-oestrogenic activities. Therefore, the structural characteristics responsible for these activities must be briefly considered.

The phenolic ring structure is typical of oestrogens, and oestrogen-like activity is produced by a number of synthetic compounds with phenolic rings. As early as the 1930s diphenyl and diphenylmethane derivatives [72] and stilbene derivatives [72–75] containing two hydroxyl groups in the *para* positions (e.g. 4,4'-dihydroxydiphenyl) were known to produce oestrogenic activity when administered by subcutaneous injection to ovariectomized rats. 4,4'-dihydroxystilbene, a hydroxylated derivative of stilbene (stilbestrol), was the first of a series of synthetic oestrogenic compounds later employed in therapy (e.g., the powerful oestrogens 4,4'-dihydroxydiethylstilbene or diethylstilbestrol [DES], hexestrol, E,E-dienestrol). These compounds may be rightly considered the first man-made xeno-oestrogens.

Phenolic ring substitutions are typical of most xeno-oestrogens, not only those that are synthetic pollutants but also natural compounds such as the phyto-oestrogens (genistein, coumestrol, biocharin A) or the resorcilic acid mycotoxins (zearalenone and zearalanol) [30].

Chlorinated compounds may also display oestrogenic activity following metabolic hydroxylation, which yields compounds containing a phenolic hydroxyl that is similar to that found in natural and synthetic oestrogens and phyto-oestrogens. The *o,p'* isomers of DDT, DDD, and DDE and the methoxy analogue of DDT, methoxychlor, have been shown the most oestrogenic of the DDT-related chemicals in uterotropic and *in vitro* assay [30]. Chlordecone, a highly chlorinated compound with reportedly weak oestrogen activity, is structurally unrelated to any of the known oestrogens.

PCBs such as the biphenylmethane compounds appear to be endowed with certain structural features that correlate with oestrogen receptor binding and oestrogenic activity (i.e., phenolic hydroxyl and chlorinating of certain carbons on the opposite aromatic ring).

In vitro, however, the oestrogenicity of *p,p'* DDE and certain PCB carcinogens is extremely weak, requiring concentrations 100,000 times greater than those of 17β-oestradiol to produce equivalent effects [27,37]. On the other hand, some biphenolic compounds are capable of inducing oestrogen-responsive β-galactosidase expression in yeast at half-maximal concentrations that are 25–10 000 fold greater than those required of DES [30].

Simple alkylphenols such as nonylphenol and bisphenol A may bind oestrogenic receptors, and they have been reported to be oestrogenic. The nature of the alkyl side chain confers some specificity, since side chains with fewer than eight carbon atoms display less oestrogenic activity [30]. Both the position (*para* > *meta* > *ortho*) and branching (*tertiary* > *secondary* = *normal*) of the alkyl group can also influence the oestrogenicity. A single tertiary branched alkyl group composed of 6–8 carbons at the *para* position on an otherwise unindered phenol ring seems to be the configuration associated with the most intense oestrogenic activity [51].

More detailed information on the relationship between structure and oestrogenicity can be found in this issue, pp. 1725–1733.

ENDOCRINE DISRUPTERS: ENVIRONMENTAL SIGNALLERS

Xenobiotic modulation of endocrine functions leading to the reduction or enhancement of hormonal effects may occur via any of the following mechanisms [13,29,39]:

a) *Direct interaction (agonism or antagonism) with hormone receptors* (due to similarities between the xenobiotic and the natural ligand) or, in the case of hormone steroid producing cells, through interference with surface receptors other than intracellular genomic receptors*.

b) *Interaction at a post-receptor level*

c) *Interference at the CNS level* (hypophyseal-gonadal, hypophyseal-thyroid, hypophyseal-adrenal axes)

d) *Interference at the level of feedback circuits*

* For example, genistein is a potent inhibitor of tyrosine kinase [5,77]. The latter can be activated by nonclassical (i.e., nongenomic) receptors for oestrogen and other steroids, which are located on cell membranes [77] .

e) *Alteration of the processes of hormone biosynthesis, release, blood transport mechanisms, biotransformation and/or elimination.*

Because of the potential complexity of their actions, which include effects on endocrine functions at a molecular level, the endocrine disrupters have been defined as 'environmental signallers' [76].

The mechanisms by which xenobiotics may interact with the endocrine system are shown in Table 3.

Table 3. Mechanisms by which xenobiotics may interact with the endocrine system

Mechanisms	Compounds	Site of action	Clinical use or toxic effects
Inhibition of hormonal biosynthetic pathways	Ethylen-2-methylsulfonate	Testis: Leydig cells	*Toxic effect*: reduced production of testosterone
	Ketoconazole	Testis: Leydig cells; Adrenals: adrenocortical cells	*Clinical use*: prostate carcinoma, Cushing disease
	Thioamides	Thyroid: prevent ormone synthesis inhibiting the thyroid peroxidase reaction to block iodine organification	*Clinical use*: hyperthyroidism
Specific cytotoxicity on endocrine structures	Mitotane (*o,p'*-DDD)	Adrenal cortex: adrenal atrophy and corticosteroid biosynthetic pathways	*Clinical use*: adrenal carcinoma (reduction of tumor mass)
	Streptozocin	Pancreas: β-cells	*Clinical use*: islet cell carcinoma
Stimulation of hormone release	Sulfonilureas	Pancreas: β-cells	*Clinical use*: treatment of NIDDM (type II diabetes)
Displacing steroid hormone from sex-hormone binding protein	Ketoconazole	Plasma proteins	*Toxic effec*: gynecomastia
Inhibition of peripheral metabolism of hormones	Aminoglutetimide	Extragonadal adipose tissue: aromatase	*Clinical use*: metastatic breast cancer
	Finasteride	Prostate and similar dihydrotestosterone dependent tissues: 5 α-reductase	*Clinical use*: benign prostatic hyperthrophy
Receptor hormone agonism	*o,p'* DDT, alkylphenols, PCBs, toxaphene, methoxychlor, dieldrin, endosulfan	Breast, gonads and similar oestrogen-dependent tissues: oestrogen receptors	*Toxic effects*: oestrogenicity, demasculinization
Peripheral hormonal metabolism increase	Chlorophenolic pesticides, organochlorines, chlorophenoxyl acids	Liver: thyroxine (T_4) metabolism increase	*Toxic effect*: thyroid function disturbances
Receptor hormone antagonism	*p,p'* DDD, vinclozolin metabolites	Prostate and similar androgen-depender tissues: androgen receptor	*Toxic effect*: anti-androgenicity, demasculinization
Hypothalamic hypophyseal-target organs control	Atrazine, formamidinic acaricides	Hypothalamic-hypophyseal-gonads axis	*Toxic effect*: reproductive function disruption
	Dithiocarbamates		

ENDOCRINE DISRUPTERS AND BREAST CANCER IN HUMANS

Debate continues over the presumed link between the presence of environmental contaminants in the body and the increased incidence of breast cancer. Since this is one of the most widely discussed and

controversial aspects of the problem of endocrine disrupters, a brief review of some of the data on this subject is in order.

DDT is known to promote the growth of oestrogen-responsive tumours [78], and *o,p'* DDT interacts with oestrogen binding protein in dimethylbenzanthracene-induced breast tumours in rats [79]. Of the 125 factors considered carcinogens or probable carcinogens for humans (most of which are chemicals), only a few have been causatively linked to breast tumours [22]. Nonetheless, long-term carcinogenesis studies have identified 160 chemicals that can provoke breast cancer in laboratory animals.

One of the most widely used approaches in the epidemiological assessment of this risk is the comparison of DDE or PCB levels in women with breast cancer and healthy controls. The results that have emerged from most of these studies indicate, in essence, a lack of correlation between blood levels of these xenobiotics and breast cancer [5,19,80,81], even in areas of high exposure where blood levels in the control subjects were also relatively high (compared to those reported in Mexico, for example [82]). The exception is the study by Wolff *et al.* [21], in which blood levels of DDE and PCBs in women with breast cancer were clearly higher than those without tumours [11 ± 9.1 ng/mL (DDE) and 8.0 ± 4.1 ng/mL (PCB) vs. 7.7 ± 6.8 ng/mL and 6.7 ± 2.9 ng/mL, respectively, in controls]. Other studies of this type are anything but conclusive [4,5,83–85].

One of the objections raised against all of the above studies is that they attempted to correlate breast cancer with blood levels of DDE or PCBs instead of the local adipose tissue concentrations (e.g., in the ductal periepithelium of the breast) [86,87], which may be 250–1000 times higher than those found in the blood [88–89]. Methodological objections have also been raised against the practice of adjusting the xenobiotic values according to lipid levels [90].

The relation between PCB exposure and breast cancer is still unclear [4,5], but it is known that susceptibility to the effects of any chemical carcinogen is generally greatest during the interval between the menarche and the first pregnancy [91,92].

It has also been pointed out that incidence rates for a given tumour may differ widely among populations exposed to similar doses of a carcinogenic agent. Indeed, susceptibility to a DNA-altering insult (or to the effects of oestrogen exposure) depends on genetic makeup, dietary factors and exposure to other carcinogens and/or tumour promoters. For example, genistein, an isoflavonoid found in soybeans, and curcumin, a component of the widely used spice, tumeric, can both inhibit the oestrogenic activities of certain pesticides [93].

The combination of a low level of 2-hydroxylation and a high level of 16α-hydroxylation of endogenous oestrogens is associated with increased risk for both breast and endometrial cancer, and many pesticides have been shown to exert a negative influence on this ratio [94]. Elevated levels of 16α-hydroxylated oestrogens have been found in mice strains that have a high incidence of malignant breast tumours [95], as well as in women with breast cancer and those with a genetic predisposition for these tumours [96,97]. However, recent findings suggest that the 2/16α hydroxyoestrone metabolite ratio is not a reliable predictor of the mammary carcinogenicity of a compound [5].

TCDD, which has anti-oestrogenic properties, might thus be expected to exert a protective effect against breast tumours in spite of the fact that it is a potent multi-site, multi-species complete carcinogen [22].

In women undergoing oestrogen hormone replacement therapy, ethyl alcohol has been shown to provoke a three-fold increase in the level of circulating oestradiol [5].

Finally, it is worth noting that the *in vitro* oestrogenicity of *p,p'*-DDE, a chlorinated organic derivative similar to dioxin, and that of certain PCB carcinogens is extremely weak, requiring concentrations 100 000 times greater than those of 17β-oestradiol to produce equivalent effects [30,37].

DETECTION METHODS

The following methods are currently available for detecting oestrogenic properties in environmental pollutants:

a) The rodent uterotropic bioassay, a time-honoured, reliable method for screening possible oestrogens [30].

b) Changes in the sexual evolution of turtle eggs. Eggs incubated at a temperature known to produce males develop as females instead due to exposure to oestradiol or environmental contaminants (e.g., PCBs) [98].

c) Oestrogen inducible strain of yeast (*Saccharomyces cerevisiae*) expressing the human oestrogen receptor. The suspected oestrogen activity is compared with that of the hormone standard based on colour changes induced in the medium that can be detected spectrophotometrically [51,76].

d) Stimulation of the formation of vitellogenin, a receptor lipoprotein expressed in all vertebrates (oviparous and ovoviviparous), which is highly sensitive to the presence of oestrogens even in males [54,99–102].

Methods used in humans are much more complex. The importance of correcting blood levels for variables such as blood cholesterol or total lipid levels has been emphasised by many investigators. It has also been suggested that xeno-oestrogens and their metabolites should be measured in adipose tissue from areas at risk (e.g., breast fat), even though this approach is more invasive. Evaluation of the 16a/2-hydroxyestrone metabolite ratio in spot urines can provide an index of the tendency toward breast cancer [94], though the value of this index is still controversial, and some insist that it is completely unreliable [5]. Some investigators maintain that the relative binding affinity-serum modified access (RBA-SMA) assay can predict the bioactivity of xeno-oestrogens in humans [103]. The same can be said for methods that tend to separate xeno-oestrogens from endogenous oestrogens in the blood and measure the oestrogen activity of extracts containing the xeno-oestrogen via bioassay [104].

Pages 1735–1745 (Structure/activity relationships) and pp. 1803–1824 (Testing of endocrine disruptors) provide more complete pictures of the *in vitro* and *in vivo* methods that are currently available for identifying compounds with oestrogenic properties.

CONCLUSIONS

The United Nation's Conference on Environment and Development (UNCED) and Agenda 21 have focused humanity's attention on the risks associated with environmental contaminants. Endocrine disrupters, together with lead and persistent organic pollutants (POPs), are undoubtedly a high-priority issue.

A resolution was approved in Ottawa in 1997 by the Intergovernmental Forum on Chemical Safety, which binds all of the Forum’s government members to promote research on endocrine disrupters and risk-prevention activities. During the discussion of this resolution, it was stressed that maximum caution must be exercised in drawing conclusions about the relation between endocrine changes and environmental contaminants. The complex picture is further obscured by the possibility of nonendocrine mechanisms in the phenomena observed (as in the case of birds of prey and DDT), the role of phyto-oestrogens and other natural products (e.g., β-sitosterol) (the case of altered reproduction in fish by pulp mills) and the impossibility of comparing data obtained in the laboratory with the environment where extremely low levels are the rule.

For example, regarding the widespread reductions in sperm counts and/or the quality of semen that have been the object of so much debate [105–109], Cooper & Kavlock [13] have pointed out that there is no direct link between these phenomena and environmental exposure to anything. Data on the incidence of breast cancer do not prove, contrary to what has been claimed, any relation between high blood levels of contaminants and increased incidence of breast tumours. A recent report indicates that Americans consume various xeno-oestrogens, anti-oestrogens and anti-androgens each day in their diets, but the net effect is activity that is for the most part anti-oestrogenic [5].

Nonetheless, we must not lower our guard [110]. While it is true that xeno-oestrogens generally appear to be less potent than endogenous oestrogens when subjected to bioassays, the persistence of these substances in the environment, their resistance to chemical and enzymatic breakdown, their slow excretion and their sequestration in fat tissue are causes for concern [24,111]. It is also true that in

embryos and foetuses the absence of a mature endocrine system increases the possibilities for adverse actions by anti-androgens and xeno-oestrogens [4]. New techniques are needed to reveal the oestrogenic or anti-androgenic capacity of these substances quickly and reliably and noninvasive tools to investigate the relation between possible endocrine disrupters and disease in humans.

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