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

# Neurotransmitters and the control of hypophyseal gonadal functions: possible implications of endocrine disruptors

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*Abstract*: The reports published so far may suggest that chemicals of plant origin or obtained by synthesis may influence the central mechanisms controlling gonadotrophin secretion through a variety of different mechanisms.

Some compounds of herbal origin seem to affect the neuroendocrine system acting as oestrogens (zearalenone and genistein). Also some synthetic chemicals seem to display an oestrogenic influence on the neuroendocrine system (the pesticide chlordecone, some of the polychlorinated biphenyls and of the alkylphenol ethoxylates). However, other synthetic molecules may affect the central control of gonadotrophin secretion acting like anti-androgens (pesticides like DDT, dioxin and vinclozolin) or by disrupting the adrenergic regulation of the hypothalamo-pituitary-gonadal axis (the pesticides chlordimeform and thiram).

These conclusions refer only to a few compounds and are largely based on indirect evidence; in many cases specific experiments are still to be performed. For many other chemicals the possible mechanism of action on the neuroendocrine system is still to be elucidated. However, the information collected so far seems to suggest that the influence exerted by environmental endocrine disruptors on the neuroendocrine system is more complex than previously anticipated.

## INTRODUCTION

Environmental endocrine disruptors pertain to many chemical categories both of natural and of synthetic origin (1–4). Many of these drugs resulted to have oestrogenic properties in *in vivo* and *in vitro* tests (5); consequently the terms ‘xenoestrogens’ or ‘environmental oestrogens’ are used as synonyms of endocrine disruptors. However, as it will be discussed below, some of the environmental ‘oestrogens’ resulted to be anti-oestrogenic or anti-androgenic and occasionally to behave as agonists-antagonists of oestrogens and progestagens; they may also have properties unrelated to those of sex steroids (see for reviews 6–8). The term ‘endocrine disruptor’ will be consequently preferred in this review unless the properties of true environmental oestrogens will be considered. Environmental endocrine disruptors may include plant and synthetic chemicals. Altogether these compounds are represented by several hundreds of molecules; only a few of them have been specifically investigated for their effects on the nervous control of the hypophyseal gonadal function. This kind of studies is made particularly difficult in view of the interactions between gonadal steroids and neurotransmitters in the control of the hypothalamo pituitary gonadal axis.

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Briefly, the two gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are released by the anterior pituitary under the control of the hypothalamic hormone luteinizing hormone releasing hormone (LHRH), frequently called gonadotrophin releasing hormone (GnRH). Gonadotrophins regulate the activity of the gonads and the secretion of their hormones (oestrogens and progesterone in the female, androgens in the male). Gonadal hormones, in turn, reach through the general circulation the hypothalamo pituitary unit whereby they can either stimulate ('positive' feedback effect) or inhibit ('negative' feedback effect) the release of LHRH and gonadotrophins (see refs 9, 10 and pp. 1633–1646 and pp. 1657–1669 for further details).

*In vivo* studies on rodents have not been able to demonstrate in a convincing way that gonadal steroids can interact with LHRH secreting neurones that do not express steroid receptors (11). The most accepted theory proposes that sex steroids interact only with neurones that directly or indirectly regulate LHRH secreting neurones through the release of neurotransmitters (12, 13). The most studied neurotransmitter is probably norepinephrine (NE): findings in rodents and monkeys suggest that NE has a stimulatory role on LHRH release. Also acetylcholine, epinephrine and excitatory amino acids basically stimulate LHRH output (12, 13). Much more uncertain is the role of dopamine (DA) serotonin (5HT) and gamma-amino-butyric acid (GABA) whose activity on LHRH neurones varies largely according to the experimental model selected (12).

More recently a long list of peptidic neurotransmitters has been reported to affect LHRH neurones; they may be either stimulatory or inhibitory on the nervous machinery leading to LHRH release. The series of stimulatory peptides includes neuropeptide Y, galanin, neurotensin and angiotensin II. Peptides inhibitory to LHRH release include opioid peptides, tachykinins, neurokinin A, neuropeptides K and gamma (13). Not necessarily, the neurones releasing the neurotransmitters listed above make direct synaptic connections with the LHRH secreting neurones: only a few of them may have direct anatomical connections with LHRH neurones; the other neurones and their neurotransmitters may act as neuromodulators of the final pathway(s) reaching LHRH secreting neurones (11–13). In conclusion the release of LHRH is the result of a complex interplay between gonadal hormones and a host of neurotransmitters that directly or indirectly may influence the activity of LHRH secreting neurones.

The interactions between sex steroids and brain neurotransmitters in the control of LHRH release may change according to the species considered: the interrelationships described above are mainly derived from the rat, but the picture is much less defined when considering human beings (14).

Studies specifically devoted to analyse the effect of endocrine disruptors on the nervous control of gonadotrophin release are rather limited. One can collect further indirect information on the central effects of these compounds through the analysis of their effects on a series of events such as sexual differentiation, sexual maturation, puberty, and sex behaviour.

It is presently recognized that in mammals the differentiation toward a male phenotype is due to the testicular secretion of the antimullerian substance and of androgens that induce a male pattern of development of the external genitalia (15, 16). Much less is known on the role, if any, of sex steroids in the development of the external genitalia towards a female pattern; probably the lack of male gonadal steroids is sufficient to induce a female differentiation, since prenatal castration of male foetuses has invariably brought to the development of female genitalia (15, 16). It has been also concluded that the neuroendocrine brain develops towards a male pattern of organization because it is exposed during the perinatal period to the presence of testosterone secreted by the testes; testosterone, however, is aromatized to oestrogens in order to induce its masculinizing effects (17, 18). In females, oestrogens do not affect the neuroendocrine brain for at lest two reasons: firstly the source of oestrogens represented by testicular androgens secreted in increasing amounts during foetal life is not present; secondly, oestrogens derived from foetal adrenals and ovaries are sequestered by alpha-fetoprotein (alpha-FP) and prevented from entering into brain cells to interact with steroid receptors (17, 18). The sexual differentiation of the neuroendocrine brain implies a series of consequences: many brain areas are sexually dimorphic and differ in size in male and females; for example the so called sexual dimorphic nucleus of the preoptic area (SDN-POA) results to be much larger in males than in females (17–20). Also the neurotransmitter distribution is different in the neuroendocrine brain of the two sexes; for example the preoptic area expresses an higher density of serotonin terminals in female rats than in males (18).

The anatomical and chemical sex related differences of the neuroendocrine brain probably provide the basis for the different patterns of the secretion of gonadotrophins and gonadal hormones in males and females at time of sexual maturation and puberty. Basically, sexual maturation is characterized by an increase of the secretion of sex steroids, predominantly testosterone in males and oestrogens in females. Very likely, these secretions are in parallel with a series of rearrangements of the neuroendocrine system that brings (1) to the readjustment at new levels of the sensitivity of brain structures to the feedback signals originating from the gonads; (2) to the loss of central restraint mechanisms to gonadotrophin release, and (3) to the activation of excitatory inputs impinging on the hypothalamo-pituitary unit (21, 22). In female mammals, the events leading to puberty are particularly evident. For the first time an hypersecretion (surge) of gonadotrophins takes place and is followed by the first ovulation. In female rodents an external marker of the occurrence of puberty is represented by the opening of the vaginal orifice, previously obstructed by a membrane (21, 22). After puberty, the secretion of the gonadotrophins will be cyclic in females, with a gonadotrophin surge preceding ovulation, and tonic in males (9, 10). Also sex behaviour is governed by the perinatal organization of the brain (17, 18) and brings about mounting behaviour in males and lordosis in females (23, 24).

The anatomical, chemical and hormonal sex related characteristics of the neuroendocrine system may be modified by perinatal endocrine manipulations. It is possible to masculinize the female neuroendocrine brain by administering to female rats testosterone during the perinatal period; conversely, it is possible to demasculinize the neuroendocrine brain by submitting male rats to orchidectomy early after birth. In rodents, the changes of the endocrine 'milieu' induced perinatally are reflected by a sex behaviour proper of the sex identity induced by hormonal manipulations (17, 18). In humans, the situation is more complex: from one side inborn errors in the secretion of sex hormones may influence the psychosexual differentiation and behaviour (25, 26); however, children born with ambiguous genitalia tend to assume the gender identity and the role behaviour according to the sex of rearing, irrespective of the genetic sex (25, 26).

In conclusion, if the perinatal exposure to some endocrine disruptor has induced some alteration in the development of the external genitalia, of the sequence of events leading to puberty, some modification in sex behaviour or in the pattern of gonadotrophin release, one may infer that also the nervous machinery controlling gonadotrophin secretion has been affected.

In the following sections of this review the possible neuroendocrine effects of environmental disruptors will be discussed. For sake of simplicity the environmental chemicals affecting the reproductive system have been grouped in two main categories: (1) all compounds of plant origin and (2) synthetic chemicals that have been classified according to the main purpose they have been synthesized for: i.e., pesticides (insecticides, fungicides and herbicides); polychlorinated biphenyls, developed for electrical equipment; alkylphenols, produced mostly to obtain surfactants; synthetic chemicals prepared to mimic the actions of sex steroids.

## PLANT DERIVED CHEMICALS

Over 150 compounds of herbal or plant origin have been identified so far which may act as endocrine disruptors (1, 27). These compounds are classically called phyto-oestrogens even if recent studies suggest that some of them may have anti-oestrogenic or progestinic effects (28, 29). A few findings may suggest that phyto-oestrogens may display direct or indirect influences on the nervous machinery controlling gonadotrophin secretion.

An indirect effect on the neuroendocrine system may be attributed to beta-sitosterol, a phyto-oestrogen found in high concentration in bleached kraft pulp mill effluent waters. When administered to goldfish this compound depresses the circulating levels of testosterone in males and of oestradiol in females; this is accompanied by an increase of plasma LH (30). These data may indicate that beta-sitosterol acts on the gonads reducing the synthesis and/or release of sex hormones: as a consequence LH secretion increases because of a lack of the negative feedback effect exerted by gonadal steroids on the central mechanisms controlling gonadotrophin secretion.

Phyto-oestrogens have been implicated as the cause of subfertility in animals grazing pasture rich in herbs producing oestrogens (31); this implies some modification of the neuroendocrine mechanisms controlling gonadotrophin secretion (see Introduction). The central effects of phyto-oestrogens seem to be supported by data by Faber and Hughes (32); these authors have reported that a neonatal treatment with genistein, a plant isoflavone, and zearalenone, a fungal resorcilic acid lactone, altered the response of serum LH to castration and to LHRH administration in adult male rats. Furthermore, neonatal treatment of female rats with the two phyto-oestrogens induced the development of the sexual dimorphic nucleus of the preoptic area (SDN-POA) according to a male pattern, i.e. it resulted much larger than in control females and similar in size to that present in untreated males (32). Phyto-oestrogens may contribute to the masculinization of the female neuroendocrine brain also by preventing oestrogens to bind to alpha FP present in female mammals: recent 'in vitro' studies show that flavonoids like genistein inhibit the binding of oestrogens to alpha FP (28). A central effect of phyto-oestrogens seems to be confirmed by a recent study by Lamartinière *et al.* (27); these authors have reported that female rats treated neonatally with genistein tend to have oestrous cycles lasting 5 rather than 4 days, with a prolongation of the phase of oestrus, an increase in the number of atretic follicles and a significant decrease of circulating levels of progesterone. All these features are compatible with modifications of the neural control of gonadotrophin release (see 10 for reviews).

Some reports suggest that also in humans phyto-oestrogens may have some effect on the neuroendocrine control of gonadotrophin secretion. It has been reported that Asian women, who traditionally are consuming a diet rich in soy products containing genistein, exhibit a menstrual cycle longer than that of Western women (33, 34); furthermore, a soy diet given daily for one month to premenopausal women significantly increased the follicular phase length by 2.5 days (35).

## SYNTHETIC CHEMICALS

An endless series of synthetic products have become part of the environment and many of them act as disruptors of the hypothalamo-pituitary-gonadal axis. As previously anticipated, these compounds have been grouped according to the main purpose they have been synthesized for.

### Pesticides

Through the years information has been collected on the effects of pesticides on the hypothalamo-pituitary-gonadal axis. Under this category have been grouped insecticides like DDT, methoxychlor, aldrin, chlordecone (kepone), mirex and chlordimeform, fungicides like thiram and vinclozolin, and herbicides like dioxins.

From a chemical point of view all these compounds fall into the category of organochlorines; however, their chemical structure as well as their specific mechanisms of action may vary largely. Consequently, not necessarily they share the same endocrine properties.

### Insecticides

DDT (dichlorodipheniltrichloroetane) is probably the most studied insecticide; it was largely used in Western countries between the 1940s and 1960s and banned at the beginning of the 1970s. DDT and its long-lasting metabolite DDE are still present in the environment, because accumulated in the biosphere, even if blood levels of these compounds are 5 times lower in the US population of the 1990s compared to the amounts present in US citizens in the 1960s (4).

The widespread distribution of DDT had a consistent impact on the wild life: it has been reported that DDT is probably responsible for an increase of the rate of feminization in western gulls of the Los Angeles area (36) and in Florida alligators (37, 38) living in DDT contaminated waters. The effects of this compound are probably due to its anti-androgenic effects. A recent paper by Kelce *et al.* (39) presents consisting evidence that DDT and DDE compete with androgens on their receptors. These in vitro data have been confirmed by *in vivo* findings showing that prepubertal male rats treated chronically

with DDE had a delay in the onset of puberty; adult male rats treated with DDE exhibited a significant decrease of the weight of the prostate and of the seminal vesicles (39). All these data may suggest that DDT and its metabolite DDE interfere with the effects of androgens both at the periphery and in the neuroendocrine brain during sexual differentiation and in adulthood.

Also methoxychlor, a compound strictly related to DDT, has been reported to induce feminization in western gulls (36). This may suggest that also this compound acts as an anti-androgen. However recent experiments have shown that a neonatal treatment with methoxyxchlor induces, in female rats, a high incidence of persistent vaginal oestrus in adulthood (40), a status that is usually a consequence of an androgenization of the neuroendocrine brain through the perinatal administration of either androgens or oestrogens (17,18) (see Introduction).

Also the neonatal treatment with the insecticide chlordecone (kepone) induces persistent vaginal oestrus in adult female rats (41). The initial proposal that chlordecone exerted its effects acting as an oestrogen and inhibiting the neuroendocrine machinery leading to the cyclic release of gonadotrophins (41) seems to find confirmation in the *in vitro* experiments by Kelce *et al.* (39); these authors have shown that this compound binds rather efficiently to oestrogen receptors even if it is able to bind also androgen receptors at higher concentrations.

Another chlorinated insecticide, mirex, has been reported to inhibit the advancement of puberty (as evaluated by the presence of ova in the Fallopian tubes) induced in prepubertal female rats by a treatment with pregnant mare serum (PMS) (42). The events believed to follow PMS administration to immature rats are follicular development and oestrogen production, facilitation by ovarian oestrogens of the release of ovulatory levels of LH (LH surge) and consequent ovulation. This sequence of events can be blocked by the administration of compounds affecting central nervous system transmitters (see 11, 12 for reviews). Consequently, one may hypothesize that mirex may affect the nervous control of gonadotrophin release. Finally, recent experiments performed utilizing the formamidine insecticide chlordimeform have shown that this compound delays by 24 hours the LH surge elicited in ovariectomized rats by an oestrogen treatment as well as the spontaneous LH surge taking place in regularly cycling female rats (43, 44). The same compound reduced also the rate of pregnancy and the litter size in pregnant rats (44). Since it has been demonstrated that this compound blocks the alpha adrenergic stimulation of the hypothalamus (45) it may be hypothesized that chlordimeform acts by disrupting the adrenergic inputs that stimulate the release of hypothalamic LHRH and of pituitary gonadotrophins (12).

It is known that all insecticides have a variety of effects on the central nervous system (46); it is then attractive to speculate that all chlorinated insecticides affect the reproductive system because they inhibit noradrenergic inputs onto LHRH neurones; however, specific experiments have to be performed to test this hypothesis. On the other hand, against this view stands the lack of effect on the reproductive system of other chlorinated insecticides (aldrin, dieldrin) that apparently do not affect the neuroendocrine control of gonadotrophin release (41).

## Fungicides

Two compounds of this group of pesticides may have some effects on the neuroendocrine control of the reproductive system: the dicarboximide vinclozolin with its metabolites, and the dithiocarbamate thiram. Pregnant rats treated with vinclozolin give birth to a male offspring with a high incidence of malformations of the external genitalia (cleft phallus, hypospadia) and of sex accessory glands. In adulthood these males mounted sexually receptive females, but only a few of them attained intromission and none ejaculated (47). These demasculinizing effects were attributed to the anti-androgenic properties of vinclozolin metabolites M1 and M2, able to displace androgens from their binding sites and to inhibit the transactivation process of the steroid-receptor complex (48, 49). In view of these properties, a central effect of vinclozolin and its metabolites in modifying sex behaviour is highly possible. It has been already mentioned in the Introduction that the neuroendocrine brain is organized according to a male pattern as a consequence of the high titres of testosterone secreted by the testes. A neuroendocrine system not receiving an adequate androgenic 'impregnation' would not deliver the proper stimuli for the expression of an appropriate male sex behaviour (see 23).

The administration of the dithiocarbamate thiram blocked the LH surge induced in ovariectomized rats by an oestrogen treatment, as well as the spontaneous LH surge occurring in regularly cycling female rats on the day of pro-oestrus (50). Thiram and other dithiocarbamates are known to suppress the activity of dopamine-beta-hydroxylase and thereby reducing the conversion of dopamine to noradrenaline (51). As for chlordimeform, one may conclude that thiram inhibits gonadotrophin secretion by suppressing the adrenergic stimulus impinging on LHRH neurones (12).

## Herbicides

Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) was identified as a contaminant of the herbicide Agent Orange and received much attention because of its toxicity. Many related compounds have been synthesized through the years giving rise to a group of molecules often considered the more toxic man-made chemicals. The dioxins represent a family of ubiquitous environmental pollutants (52) and a series of toxic effects have been attributed to TCDD and related compounds (4, 52). Effects on the reproductive system have been also reported. Studies in fish demonstrate that animals grown in bleached kraft pulp mill effluent waters contaminated with TCDD exhibit low circulating titres of gonadotrophins, testosterone and 17,20beta-dihydroprogesterone, and a reduced response of LH to LHRH; finally, female fish fail to ovulate under LHRH treatment (53). This series of data may suggest that TCDD affects the nervous control of gonadotrophin release. This hypothesis seems to be confirmed by experiments performed in rats. TCDD administered to adult male rats decreases plasma levels of LH, testosterone and 5alpha-dihydrotesterone and inhibits testicular steroidogenesis (54–57). TCDD is known to be transferred *in utero* and during lactation from the mother to the young (58, 59); it is then possible that exposure *in utero* to dioxin may affect the male sexual differentiation and behaviour. Ad hoc studies have been performed by two groups of investigators (60–63). From their findings it resulted that the treatment of pregnant rats with TCDD brings about a reduced secretion of LH and androgens in adult male offspring (60, 61) and reduces the weight of male sex accessory glands and spermatogenesis (62, 63). In spite of these modifications no impairment of male fertility was observed (62, 63). Some subtle modifications of the neuroendocrine brain were however present in male progeny exposed to TCDD when a few studies on their sex behaviour were performed. When castrated as adults and treated with oestrogens and progesterone, these animals displayed a female type of lordosis quotient when mounted by males; this feature was only slightly expressed in castrated-steroid primed male rats, not exposed to TCDD during their foetal life. Furthermore, when TCDD exposed castrated-steroid primed male rats were challenged with LHRH they responded with an LH surge similar to that induced in ovariectomized-steroid treated female rats; this kind of surge was absent in castrated-steroid primed male rats born to mothers not treated with TCDD (61). Collectively, these findings strongly suggest that TCDD impairs sexual differentiation in male rats. It remains for further studies to clarify whether the demasculinization and feminization of male progeny is due to a direct effect of dioxin on the neuroendocrine system or is an indirect one, by depressing androgen production in the foetal testis and consequently preventing the male organization of the neuroendocrine brain.

The effects of TCDD in female rats have been less studied. The findings of Gray & Ostby (64) indicate that an *in utero* treatment with this compound induces in female rats a delay of puberty, with persistence of a membrane ('thread') on the vaginal orifice and the presence of a phallus with a complete to partial cleft; a large number of TCDD treated female animals exhibited a persistent vaginal oestrus as adults, fertility was reduced and at autopsy a slight reduction of the weight of the ovaries was observed. Also these features could be attributed to an alteration of the neuroendocrine brain: of particular significance may be considered the delay of puberty and the high incidence of persistent vaginal oestrus, which could be based on a failure of the central mechanisms in inducing the release of appropriate amounts of gonadotrophins at the time of puberty, and in eliciting the cyclic release of gonadotrophins in adulthood.

### Polychlorinated biphenyls

Organochlorines related to dioxins are polychlorinated biphenyls (PCBs) which may have or have not dioxin-like activity; for example, PCBs submitted to extreme heat are converted to dioxins (4). PCBs have been produced until the '70s in large amounts for use in electrical equipment and other applications. PCBs are ubiquitous and persistent environmental contaminants and have been reported to bind to oestrogen receptors (65). Studies in turtles—where sex determination is dependent upon temperature have shown that eggs exposed to PCBs produced only females even if incubated at a temperature that would produce males (66). Once again, one may hypothesize that shifts in sexual differentiation may include modifications of the neural control of the hypothalamo pituitary gonadal axis. This hypothesis seems confirmed by experiments performed in minks fed with fish from PCBs and dioxin polluted areas. This kind of diet reduced food intake, induced a poor reproductive performance and a low survival rate of the kits (67). Of particular interest is depression of food intake: it is known that in rodents the control of appetite and weight is localized in the central nervous system where a major role is played by the hypothalamus and by the nervous pathways impinging on it (68). On the other hand, it is known that deficiency of nutrients and in particular a caloric imbalance in nutrients assumed may alter the hypothalamic control of reproduction (69, 70). Consequently, one may hypothesize that in these minks the neuroendocrine control of reproduction had been affected by the diet administered.

### Alkylphenol ethoxylates

Alkylphenol ethoxylates (APEOs) are basically non-ionic surfactants used as industrial and agricultural cleaners or as detergents and emulsifiers for many products ranging from household cleaners to oils. They are being used for over 40 years and result among the most diffused polluters. Recent concerns about their presence in waters and sediments in Europe has brought to their removal from cleaning products. However, still in 1990, 450 millions pounds were produced in the United States; it has been estimated that over 300 000 tons of APEOs are produced in the world every year (see 71, 72, for reviews). These chemicals resulted to bind to oestrogen receptors (5, 73) and to be oestrogenic in fish and rats; therefore APEOs, like oestrogens, induce in male trout the synthesis by the liver of vitellogenin; this protein is taken up by the oocytes of female trout and is responsible for the growth of oocytes in the month preceding ovulation (72, 73). One may hypothesize that this shift towards a female kind of synthesis could be parallel to some sort of feminization of the fish neuroendocrine system.

Central effects of APEOs may be inferred through the analysis of some studies performed in rats. Sharpe *et al.* (74) have reported that the perinatal treatment with a variety of APEOs (octylphenol, OP; butylbenzylphtalate, BPP and octylphenol polylthoxylate, OPP) induces in male rats a reduction of testicular size and of daily sperm production. Also in adult male rats the chronic administration of OP brings about a series of modifications of the genital tract, such as shrinkage of the testes and of male sex accessory glands, as well as disruption of spermatogenesis with increased incidence of sperm deformities (75, 76). All the effects observed in male fish and rats may be attributed to the oestrogenic effects of APEOs. However, the modifications of the genital tract were probably accompanied by some effects at the level of the neuroendocrine system, since a prolonged administration of OP brought about a decrease of the circulating levels of gonadotrophins and testosterone and reduced food intake in treated animals (76).

Octylphenol disrupts the control of gonadotrophin secretion also in female animals: adult female rats treated neonatally with OP exhibit in a large percentage persistent vaginal oestrus. However, the central effects of OP, if any, do not bring about an increase in the size of the SDN-POA (77) as other oestrogenic compounds do (see the section devoted to ‘plant derived chemicals’); this does not exclude that OP may interact with some other hypothalamic or extrahypothalamic area.

### Synthetic steroids—The case of diethylstilbestrol

Steroidal compounds are continuously manufactured by the pharmaceutical industry to serve the most different uses. Many of these contribute to environmental pollution when they reach the sewer system,

either under the form of the original molecules or as metabolites that still may have biological effects (5). Probably diethylstilbestrol (DES) has been the most studied synthetic steroid for the impact it had on human health. DES was prepared in the 1940s as the first synthetic oestrogen and was used until the 1970s; it was mainly used as an oestrogenic aid to avoid miscarriages during pregnancy (for reviews see 78). It has been estimated that 5 to 10 million Americans were prescribed DES during pregnancy (DES mothers) or were exposed to the drug *in utero* (DES daughters and sons) (79). Harmful effects of DES became evident at the beginning of the 1970s and the drug was banned by FDA in 1971. However, the steroid is still used in selected cases, like the treatment of advanced prostate cancer, as a morning-after pill in emergency situations and in cattle feed to promote growth (5). Consequently DES still contributes to environment contamination.

Retrospective studies indicate that a high percentage of DES daughters has malformations of the genital tract, not necessarily linked to a decrease of fecundity, but related, however, to high risk for a not favourable pregnancy outcome (78). Also DES sons exhibit an higher incidence of malformations of the genital tract than unexposed controls; however, they result as fertile as unexposed men (80). Nothing certain is known on the possible central effects of DES in humans. Studies in experimental animals may suggest that *in utero* exposure to DES may affect the neuroendocrine system. It has been reported that high doses of DES given perinatally induce in female rats a significant enlargement of the SDN-POA (32, 77), a typical central effect of oestrogens already discussed in previous sections. It has been also reported that *in utero* treatment with DES brings about in infant male and female monkeys a modification of the secretory patterns of FSH and LH (81). Also these results may suggest a central effect of DES.

## CONCLUSION

The general picture coming out from this review suggests that environmental endocrine disruptors may indeed affect the nervous control of gonadotrophin secretion. However, most of the conclusions have been based on indirect evidence; only in a few cases specific experiments have been done. Even in the latter case the hypotheses presented are far from being conclusive for different reasons:

**1** Laboratory experiments are short lasting in comparison to the life long exposure of wild and human beings to xenobiotics.

**2** Obviously, the studies are directed to evaluate the effect(s) of a single chemical on the parameter under investigation; however animals and humans are continuously exposed to a ‘sea’ of endocrine disruptors whose cumulative effects are far from being clarified.

In particular only a few studies have been devoted to the effects of a mixture of chemicals on the endocrine system. This is becoming particularly interesting in view of recent data showing that: (a) mixtures of xenobiotics develop a cumulative oestrogenic effect (82); and (b) also molecules with negligible oestrogenic like effects achieve a consistent potency when they interact with oestrogenic receptors in combination (83).

**3** In most cases the studies have been addressed to analyse the effects of an endocrine disruptor in one species. Obviously the conclusions derived from that species not necessarily apply to others. Studies devoted to ascertain the effect(s) of the same chemical in a large series of species are needed. In particular very little is still known about the impact of endocrine disruptors on the reproductive system in humans. However, some evidence suggests that the constant exposure to environmental endocrine disruptors may be harmful to human reproduction (see pp. 1685–1701).

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