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ENDOCRINE DISRUPTORS, THE ENVIRONMENT AND HUMAN DISEASES

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Background

Environmental contaminants are increasingly common. These contaminants include endocrine disruptors, which pollute the soil, water, air and land. They also affect the food chain and can be a risk for human health, making them a problem which must be addressed in public health and prevention programmes. This issue also has repercussions in terms of biosafety.

This document has been prepared by the Italian Committee for Biosafety, Biotechnology and Life Sciences following discussions among its own members and with the contributions of external experts, to provide both the basis and an up-to-date description of the issue to meet the needs of both the committee itself and external organisations.

Prof. Anna Maria Colao, Prof. Andrea Isidori and Prof. Silvia Migliaccio, experts from the Italian Endocrinology Society[1], contributed to the preparation of this document through their participation in the plenary meeting of 3 October 2016.

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[1] Società Italiana di Endocrinologia

Introduction

Hippocratic tradition emphasises the environmental causes of disease and the need for harmony between individuals and the natural environment as the correct philosophy for maintaining good health. The general population is now well informed about environmental pollution and the increased risk of cancer. However, there is still little public awareness of how other common major diseases are affected by environmental and lifestyle changes. The good news is that there is a constant increase in reliable scientific studies helping to clarify the pathophysiological mechanisms underlying the association between numerous non-transmissible chronic diseases and environmental pollutants (not just in relation to the risk of cancer), although there is still much to learn (1).

Endocrine disrupting chemicals (EDCs), also known as endocrine disruptors, comprise a vast category of chemicals and chemical mixtures which affect the function of the endocrine system, causing adverse effects to the health of organisms or their offspring. They can bind as agonists or antagonists to the receptors of various hormones, such as steroid or thyroid hormone receptors, or interfere with them in various ways and through different mechanisms, through the synthesis, secretion, transport, binding, action upon and elimination of such hormones in living beings.

EDCs include **aromatic polycyclic hydrocarbons, benzene, dioxin, phthalates, perfluorocarbons, bisphenol A (and octylphenols and nonylphenols)**. These compounds can cause severe injury (although this might not be immediately obvious, as minimal doses do not cause acute toxic effects), and in the case of largescale environmental exposure, can produce population effects with ecological repercussions. This disruption can cause birth defects and other developmental disorders. EDCs are above all known to cause learning difficulties, severe attention deficit disorders, cognitive and brain development disorders, physical malformations, sexual development problems, feminisation of males and androgenisation of females.

They also have a hormonal action. EDCs may be found as environmental pollutants, such as in the case of numerous organic halides including polychlorobiphenyls (PCBs) and similar compounds classed as persistent organic pollutants, as natural components of some foods, such as the phytoestrogens in soya, or as contaminants, such as bisphenol A (BPA) derived from plastics or various types of agrochemical.

Major classes of endocrine disruptor include the various types of chlorinated chemicals, which are often carried in the atmosphere for long distances, and phenolic compounds. The environmental transmission of these compounds has been amply studied, with investigation of both the transport of various stable compounds, such as organic halides, and their repercussions for colder areas where global atmospheric circulation causes them to recondense, producing biological effects on various organisms (2).

Current knowledge and activities pertaining to endocrine disruptors can be summarised as follows:

A) In animals, endocrine disruptors can act on the hormone system, affecting reproduction. A cause-effect relationship has been demonstrated in wildlife as well as in laboratory animals; it has not yet been demonstrated that endocrine disruptors in the environment can affect reproduction in wild animals.

B) An increase in cases of reproductive disorders, some types of cancer, metabolic diseases such as obesity and diabetes, and cardiovascular diseases (1,3) has been associated with the presence of endocrine disruptors. While the results seem to be consistent with the thesis that EDCs are to blame, it has not been possible to document a causal relationship between exposure to a substance with endocrine activity and an effect on the human body (4).

C) Further research is needed to perform a full risk assessment, especially with regard to the toxic effects of low concentrations and the “cocktail effect” of these chemicals.

D) Some known endocrine disruptors are already regulated by legislation for reasons other than their hormone activity (general toxicity, carcinogenicity, reproductive toxicity) (5).

Given their complex nature, exposure to EDCs can result in numerous clinical phenotypes: investigation of the mechanisms of action is a constantly evolving field of research. The highly varied nature of these compounds impedes the identification of a common mechanism of action. While some substances have some similar properties (molecular weight, or the presence of specific highly reactive groups), no common property can be identified for all endocrine disruptors, so it is impossible to generalise - and much less predict - the exact mechanism of action. However, this vast class can be divided into three broad categories:

1) Hormone agonists, the intake of which leads directly or indirectly to a type of receptor activation known as hyperstimulation. Phytoestrogens and thyroid-stimulating chemicals fall in this category.

2) Hormone antagonists, which interact with hormone receptors and directly or indirectly impede their physiological activation (inhibition). This category includes anti-oestrogens and anti-androgens.

3) Metabolic modifiers, which interfere with normal endogenous hormone secretion or with other steps in normal hormone activity, including their transport in the blood, intracellular pre-processing and post-processing, thus affecting their decomposition and elimination. Chemicals which stimulate liver metabolism or which are able to chelate circulating hormones can be included in this category.

Although there have been significant advances in this field, worldwide research into EDCs has not yet led to incontrovertible scientific evidence. Many of these compounds have short half-lives, others have low molecular weights, others still act through metabolites; their identification is therefore far from simple and requires both suitable instrumentation and a specialist clinical background. There is also the legitimate concern that many studies are highly compromised, whether due to the authors' positions or the source of their funding.

The main critical points pertaining to the study of EDCs are as follows:

1) Delayed effects In many cases, EDC intake does not have immediate effects. For example, pre-pubertal or intrauterine exposure may affect fertility many years later, and the underlying cause of numerous diseases in adults might be intrauterine exposure to endocrine disruptors. Epigenetic effects, such as in the case of DNA expression modulation through methylation, may be transmitted from one generation to the next, sometimes without any apparent clinical signs in the subjects directly exposed. The effect may be delayed by as much as 20-30 years, as documented for some cancers (cervical and testicular cancer), or even longer, as has been postulated for some forms of dementia.

2) Multiple exposure Most studies of endocrine disruptors aim to identify the effects and mechanism of action of a single substance. Possible interactions between the various chemicals are largely ignored. However, given the heterogeneous nature of EDCs, their distribution should be considered as ubiquitous and concomitant exposure to multiple compounds is highly likely. It is possible that the mechanisms of action of multiple EDCs are additive or even synergic, leading to more marked effects from a more limited exposure to the individual components.

3) Dose-response dynamics Endocrine disruptors rarely present linear dose-response dynamics. U-shaped or inverted U-shaped response curves have been seen in many cases, while during certain periods of development, exposure may give rise to clinical signs regardless of the dose. Lipophilic chemicals tend to accumulate in the adipose tissue, leading to their gradual release over time and the possible onset of clinical signs years after the original exposure ended. EDC metabolites often have a higher biological activity or accumulation capacity than the original compounds themselves.

4) Measurement of effects The extreme heterogeneity of endocrine disruptors makes it difficult to estimate their effects exactly, even when there are clinical signs which can be attributed to them. Furthermore, long-term exposure to low doses often makes it difficult to establish the causal connection between the culpable agents and the clinical effects. Each individual will have a unique exposure pattern to both known and unknown EDCs, and physiological differences in their metabolism and the subject’s body composition will alter their half-lives and biological effects. Traditional

toxicology methods and the many animal studies offer only a partial picture of the effects of EDCs.

5) Limitations of translational models Animal models are commonly used to evaluate the effects of various substances in vivo. However, it is difficult to estimate how well the results can be extrapolated to humans, due to temporal dynamics as well as other variables. Furthermore, studies aiming to identify the effects of individual substances are unlikely to represent reality: simultaneous exposure to a low concentration of multiple chemicals is not feasible in the laboratory. In addition, some substances may be inert if studied in isolation but biologically active in the presence of other chemicals or in complex biological matrices such as blood. In any case, despite these limitations animal models are still the most reliable method for studying endocrine disruptors.

6) Clinical dimorphism The severity of clinical signs caused by exposure to the same mixture of EDCs may show gender differences. For example, exposure to oestrogen-like agents can cause premature thelarche in girls, while in boys it can lead to gonadal dysgenesis. This is partly due to physiological differences in the interaction between the hormone and receptor; however, it can also be hypothesised that the different clinical presentations may be due to the different expression of the enzymes involved in the metabolism of endocrine disruptors. The extent of the dimorphism also depends on whether females are in the pre-pubertal, reproductive or menopausal age.

On the basis of these observations, and given the concern generated by EDCs, the Italian Ministry of the Environment has produced a guidebook for the public.

The European Union has named 564 chemicals as known or suspected endocrine disruptors. Of these, 147 may be persistent organic pollutants or produced in large volumes; just 66 are proven to act as endocrine disruptors (category 1) while there is only limited evidence that another 52 are potential endocrine disruptors (category 2) (5).

Last but not least, there is still a general lack of evidence as to the acute and chronic effects of environmental pollutants and endocrine disruptors in relation to gender, and therefore a need for studies of populations of comparable age and sex.

Endocrine disruptors and thyroid cancer

The incidence of thyroid cancer is progressively increasing worldwide. In Italy, it is the fifth most common cancer in men and the second most common in women under 50 (6). The increasing incidence is evident not only in western countries (7) but also in the so-called emerging countries (8); moreover, this trend shows no signs of slowing down even in countries where diagnostic ultrasound has long been widely used (9). Taken together, this all suggests the possible aetiopathogenetic role of environmental pollution, which is also progressively increasing, in the development of thyroid cancer. However, despite our in-depth knowledge of the genetic mutations associated with this disease (10), the causal factors are still largely unknown.

Thyroid function is intrinsically correlated with environmental exposure. The thyroid gland needs exogenous iodine, a natural trace element, to synthesise the thyroid hormones. However, the gland's function is also influenced by a wide range of synthetic compounds (e.g. phthalates, dioxin and alkylphenols) (11). These compounds form part of a diverse group of synthetic chemicals deriving from human - and above all industrial - activity, known as endocrine disruptors. They are capable of causing long-term environmental contamination, and have a high probability of entering into contact with human beings (12). They interfere with various aspects of thyroid hormone function: their metabolism (by blocking the intracellular uptake of iodine and/or altering its organification); their transport (by affecting their binding with plasma proteins and uptake in the peripheral tissues); and their transcriptional activity (by affecting their binding with receptors and their cofactors) (13).

There is still limited scientific evidence of the association between environmental pollution and the development of thyroid disease. To date, we only have data on the impact of exposure to certain categories of pollutants on the thyroid hormone profile

and the development of autoimmune thyroid diseases (14). Although endocrine disruptors are thought likely to have a role in the development of thyroid cancer, this theory is not yet supported by sufficient evidence.

The most widely accepted pathogenic hypothesis is that exposure to thyroid-disrupting chemicals may induce the neoplastic transformation of thyrocytes through their hyperstimulation, due to excessive pituitary TSH production as a consequence of the inhibited gland function (13). However, genetic screening of thyroid tumours in subjects from some highly polluted areas of China and Italy (15,16) revealed a higher than expected prevalence of the mutational event $BRAF^{V600E}$, suggesting a possible direct mutagenic action of environmental pollutants (17).

The few epidemiological studies of the association between environmental exposure and thyroid cancer have almost all investigated occupational exposure. A Swedish and a Chinese study both demonstrated a significantly higher incidence of thyroid cancer in workers (in the footwear and textile industries respectively) exposed to chemical mixtures (18), suggesting a link between chemical contamination and the development of this disease. However, epidemiological data focusing on a single category of pollutants are unavailable.

To date, the only experimental evidence of the possible role of endocrine disruptors in thyroid carcinogenesis derives from studies of animals exposed to high doses (19); there is no information regarding the possible carcinogenic effect of exposure to low doses, the typical model for human beings exposed to environmental pollution.

Endocrine disruptors and pituitary tumours

A handful of small-scale epidemiological studies have suggested that the environment may have a role in the pathogenesis of acromegaly. Cannavò et al. (20) assessed the prevalence of acromegaly in 4 areas in and around Messina in Sicily: Area A (Ionian area), Area B (Tyrrhenian area), Area C (Messina city) and Area D (5 highly industrialised towns in the province of Messina). Taking Area A as the control, it was calculated that the population in the industrialised area (D) had a higher risk of

developing acromegaly than the other areas. The Italian Regional Environmental Protection Agency[2] identified the following atmospheric pollutants in Area D: non-methane hydrocarbons, benzene, toluene, 1-3-butadiene, trans-2-pentene, 1-2-3-tribenzene, cis-2-butadiene, trans-2-butene, 2-methyl-1-pentene, acetylene, α-pinene, β-pinene, cyclohexene, ethane, isobutene, isopentane, isopropylbenzene, met-cyclo-pentane, m-xylene, n-decane, n-nonane, p-diethyl-benzene, o-ethyltoluene, n-pentane and n-octane.

Pesatori et al. (21) conducted a study to assess the incidence of sporadic pituitary tumours in the area of Seveso, Italy, which was affected by an industrial accident at the company ICMESA[3] in the town of Meda in 1976 which caused the release and dispersal of a cloud of the dioxin TCDD. The incidence of sporadic pituitary tumours was assessed in three areas of Seveso associated with low, medium and high dioxin exposure, with no significant differences found.

The aryl hydrocarbon receptor pathway seems to be the connection between pituitary tumours and the environment. The most powerful ligand of the aryl hydrocarbon receptor is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The other ligands include more than 400 exogenous compounds, including polycyclic aromatic hydrocarbons such as benzo[a]pyrene and PCBs (22). Genetic variations interfering with the aryl hydrocarbon receptor signal pathway in acromegalic patients living in highly contaminated areas are associated with larger adenoma size and resistance to treatment with somatostatin analogues (23).

Endocrine disruptors and obesity

Endocrine disruptors alter the homeostasis of the endocrine and metabolic system through their ability to mimic and/or antagonise the biological activity of the hormones produced by the body. Discussion of the concept of contaminant over the years has led to the universal acceptance of the following definition from the *Codex Alimentarius*:

[2] Agenzia Regionale Prevenzione e Ambiente
[3] Industrie Chimiche Meda Società Azionaria

"*Any substance not intentionally added to food or feed for food producing animals, which is present in such food or feed as a result of the production…[and] manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or feed, or as a result of environmental contamination.*"

It is clear that endocrine disruptors can enter the food chain in various ways. The chemical properties of these compounds enable them to interfere with normal intracellular signal transmission processes, meaning they may have an important role not only in the induction of carcinogenesis but also in the aetiopathogenesis of endocrine and metabolic disorders (24).

Another property of these compounds, in contrast with other environmental toxins and chemicals, is that their endocrine disrupting effects are often induced at small doses and depend on the period of exposure. Apparently low exposure levels thus often cause molecular and cellular changes which, although minimal, can induce more serious pathophysiological effects with prolonged exposure. It is also important to remember the potential persistence of these compounds in the adipose tissue, with the risk of a weak but persistent effect on the target tissues (25). Their effects are often pleiotropic and exposure to endocrine disruptors has been correlated with infertility, delayed puberty, premature birth and the development of various diseases, including cardiovascular and neoplastic diseases and skeletal disorders (1, 13, 26-29).

In recent years, numerous studies have also revealed possible correlations between exposure to environmental EDCs and the development of metabolic syndrome, type 2 diabetes mellitus and obesity (24,30). Recent decades have seen a dramatic rise in obesity, in children and adolescents as well as in adults (31). Obesity is a clinical condition which carries an increased risk of developing chronic metabolic disorders (such as type II diabetes mellitus), cardiovascular diseases and neoplastic diseases, as well as an increased risk of bone fractures. It is characterised by increased size (hypertrophic obesity) or number of adipocytes (hyperplastic obesity). Numerous factors have a role in the aetiopathogenesis of this condition, due to the complex interactions between genetic, behavioural and environmental factors. Endocrine

disruptors are included among the factors potentially involved in the development of metabolic disorders such as type 2 diabetes mellitus and obesity (32).

The main chemicals which act as obesogenic endocrine disruptors include bisphenol A (BPA), tributyltin (TBT), diethylhexyl phthalate (DEHP), nonylphenol (a synthetic alkylphenol), genistein, phthalates, perfluorocarbons (PFC) and perfluorooctanoic acid (PFOA) (33-36). Obesogens induce changes which interfere with the normal function of hormones (e.g. oestrogens, glucocorticoids) and of factors such as leptin, ghrelin and neuropeptide Y, as well as acting through the inhibition of the enzyme aromatase or interference with the expression of receptors for steroid hormones, retinoic X, PPAR gamma and glucocorticoids (37,38). Recent studies also found a direct effect on adipocyte differentiation and the induction of dysfunctional adipose tissue (39-41). In animal models, it has been demonstrated that long-term exposure to low doses of BPA (hence in a manner similar to environmental exposure) can affect adipocyte differentiation, increasing the proliferation of preadipocytes and causing the early expression of the genes involved in glycolipid metabolism (40). This induces the formation of hypertrophic adipocytes and alters the insulin-dependent signal, increasing the expression of pro-inflammatory cytokines. BPA has also been implicated with the onset of polycystic ovary syndrome and type 2 diabetes mellitus, both of which are characterised by insulin resistance (42-43).

Given the literature evidence, it is reasonable to suppose that obesogens can predispose individuals to weight increase through the mechanisms described above and through changes induced to the metabolic set-point, especially if exposure took place during particularly vulnerable periods such as the first years of life (24). In fact, even if exposure to endocrine disruptors may occur in all stages of life (from conception and fetal development to senescence), the changes to the endocrine system caused by these chemicals are more critical if the exposure takes place during prenatal or early postnatal life, due to the rapid changes in the body's growth.

Data from experimental animal models suggest that exposure to obesogenic endocrine disruptors may induce the reprogramming of cell differentiation due to epigenetic

modifications (29,44). In cell models developed to study adipogenic differentiation, some of the genes which effectively regulate pre-adipocyte proliferation and promote adipocyte differentiation were identified. Of these, the proliferation of PPAR-gamma is fundamental. The activation of PPAR-gamma leads to gene expression changes which favour the differentiation of adipocytes and the storage of energy. The persistent activation of the PPAR-gamma-dependent signal due to dietary "overload" or the action of endocrine disruptors disturbing the system may be obesogenic (37,45), thus performing an important role in the aetiopathogenesis of obesity as well as of type 2 diabetes mellitus.

Endocrine disruptors and fertility

EDCs and prenatal development

It was once thought that EDCs acted only through nuclear receptors for oestrogen, androgen, progesterone and thyroid hormones and retinoid receptors. However, recent studies have shown that their mechanism of action is not that simple. Endocrine disruptors exert effects on the endocrine and reproductive system through nuclear receptors, non-nuclear steroid hormone receptors (membrane oestrogen receptors), non-steroid receptors (receptors for neurotransmitters such as serotonin and dopamine, norepinephrine receptors), orphan receptors (aryl hydrocarbon receptors), enzyme pathways involving the biosynthesis and/or metabolism of steroids and numerous other mechanisms (46).

Unlike adults, infants and children are exposed not only to toxic chemicals in the environment but also indirectly, during intrauterine life, to any toxins which accumulated in their mother before her pregnancy. Hundreds of toxic chemicals can directly affect the fetus through the umbilical cord and the placenta. These include countless neuroimmune and endocrine toxins which may affect the critical phases of hormonal, neurological and immunological development. Given their lipophilic structure, EDCs can easily cross biological barriers such as the placenta and blood-brain barrier, for which reason they have been categorised as "poisons without

passports". Both human and animal studies have demonstrated that the effects of endocrine disruptors on the offspring are not limited to congenital abnormalities or reduced IQ but years later can induce behavioural and health changes persisting throughout life, even in initially healthy offspring (47).

It has been amply demonstrated that prenatal exposure to EDCs can cause a series of disorders correlated to systematic abnormalities such as (48):

- → Growth disorders, with abnormal development of the brain, heart, breasts and sex organs (undescended testicles, micropenis and labial fusion);
- → Central nervous system developmental disorders;
- → Immune system developmental disorders.

These disorders may also give rise, through cumulative effects, to more complex organ and system changes and dysfunctions, impairing cross-talk between the immune, nervous and endocrine systems (psycho-neuro-immuno-endocrine axis).

Effects on male fertility

The male reproductive system may be significantly affected by the intake of endocrine-like substances from fetal age (49). Anti-androgen chemicals were among the first for which there was documented evidence of the effects of EDCs, and may offer a typical model.

Clinical data from human males and animal models have identified an association between endocrine disruptors and gonad development disorders, collectively known as testicular dysgenesis syndrome (TDS), which includes conditions such as cryptorchidism, hypospadias, micropenis, in situ testicular carcinoma, infertility and predisposition to the development of testicular tumours (50). TDS can be attributed to impaired embryonal development of the Sertoli and Leydig cells, affecting the germ cell compartment. Anti-androgens, xeno-oestrogens and dioxins are the most extensively studied endocrine disruptors in relation to male reproductive health, but the list of chemicals with potential effects on fertility and the urogenital tract is constantly increasing. The results of a recent meta-analysis suggest that the role of

individual endocrine disruptors is probably less important than was supposed, while the cocktail effect of exposure to multiple agents is more likely to be the cause of significant changes (51).

Some authors believe that the modification (reduction) of secondary sexual features (androgenisation) in the male populations of western countries (especially in young adults), which is reflected in measurable phenotypic changes (height, ratio of limb to trunk length, body hair, total number of sperm produced), can be attributed to an evolving phenomenon (loss of reproductive advantage), while others believe it to be due to a progressive "oestrogenisation" linked to dietary factors (additives and phytoestrogens) or pollutants (EDCs).

Urogenital tract abnormalities

The male gonads normally begin to develop during embryogenesis. The processes enabling the further development of the mesonephric ducts and the involution of the Müllerian ducts take place under the stimulation of testosterone and anti-Müllerian hormone respectively. Endogenous hormones are also involved in the development of the external genitals (testosterone [DHT]) and in testicular descent into the scrotal sac (insulin-like peptide 3 [INSL-3]). Exposure to agents with anti-androgen properties during fetal life can lead to various clinical phenotypes, from hypospadias to cryptorchidism.

An association between parental exposure to pesticides and the onset of hypospadias in neonates was found in a meta-analysis (52), but some more recent studies did not confirm these initial data, although they did suggest a possible link with maternal exposure to heavy metals or EDCs other than pesticides (53). Associations with other agents such as PCB and PBB were not statistically significant in studies in humans. However, the small sample size, due to the rareness of hypospadias in the general population, limits the statistical power of these studies (12). Studies of animal models have led to the identification of a number of endocrine disruptors capable of causing hypospadias. Some, such as phthalates, act by inhibiting the production of endogenous

testosterone, while others, like some fungicides and pesticides, block androgens from binding to their receptor. Exposure to multiple substances leads to synergic effects which exponentially increase the risk of hypospadias (54).

The observation that the rate of cryptorchidism does not differ significantly between monozygotic and di- or trizygotic twins and triplets suggested an aetiopathogenetic role of the placental endocrine milieu. High pesticide concentrations have been found in the adipose tissue of cryptorchidic subjects and in the milk of their mothers (55), while data for biphenyls are less conclusive. High dioxin levels in maternal milk have been associated with a greater risk of cryptorchidism, but no similar correlation has been seen with placental levels (56). The anti-androgen action of phthalates produces a diminished testosterone concentration and high levels of LH, possibly explaining the correlation between exposure to these compounds and an increased risk of cryptorchidism (57).

Both cryptorchidism and hypospadias are known risk factors for testicular tumours, especially germ cell tumours (50). Studies in humans did not find higher biphenyl concentrations in subjects with testicular tumours, but their concentration in the serum of the mothers of men with germ cell tumours was significantly increased, compatibly with a transgenerational effect.

Of the other EDCs likely to be involved in the aetiopathogenesis of testicular tumours it is worth mentioning pesticides such as DDT and its metabolite dichlorodiphenyldichloroethylene (DDE), which although unused for more than 40 years are still present in the food chain and found in the tissues of various animals (12).

Impairment of spermatogenesis

Recent decades have seen the publication of conflicting scientific evidence on the supposed decline of sperm quality worldwide. Environmental factors and exposure to EDCs have been cited by authors asserting the existence of this decline, while detractors have pointed to errors in the selection of the caseloads and the different methodologies used. Experimental evidence of the effects of endocrine disruptors on the production of male gametes in both adult life and in the developmental age has

been obtained from animal models; following in utero exposure, EDC acts on spermatogenesis both directly and through transgenerational effects.

As amply discussed elsewhere, the effects of EDCs are often only seen years later. For this reason, transversal studies in humans are unable to provide incontrovertible scientific evidence of the correlation between exposure to one or more EDCs and impaired spermatogenesis. There is conflicting literature evidence of the correlation between impaired semen parameters and exposure to pesticides, PCBs and surfactants in adulthood. Some studies found deleterious effects on semen quality in exposed subjects, while others were inconclusive (5, 59-60). In contrast, intrauterine exposure to compounds such as diethylstilboestrol, PCBs, dibenzofuran and dioxins seems to have indisputable effects on all semen parameters (61-62). These data give weight to the hypothesis that the effects of EDCs are exponential or dominant on fertility when exposure occurs during intrauterine life, especially during gonadal morphogenesis (during the Sertoli cell proliferation phase).

Effects on female fertility

As with males, the female reproductive axis can also be affected by exposure to endocrine disruptors from fetal life onward (49). EDCs can change both the structure and the function of the reproductive organs. However, the literature evidence does not enable definitive conclusions to be drawn: the mechanisms of action are not yet fully known, and the list of potential endocrine disruptors is growing at an alarming rate.

Changes to ovarian function

In animal models, intrauterine exposure to BPA, pesticides and phthalates has been associated with the development of fewer primordial follicles (5, 63) following epigenetic mechanisms which change the expression of the genes involved in meiosis. After birth, exposure to the same substances (especially at high doses) accelerates follicle transition, with the recruitment of a greater number of primary follicles, leading to rapid follicular atresia (64) with an associated drop in oestrogen secretion (65). Other endocrine disruptors, such as parabens, some fungicides, tributyltin and

phytoestrogens, act at various levels on folliculogenesis, inducing atresia or reducing the number of lutein bodies and increasing the number of cystic follicles (66-67). There is an almost total lack of experimental data in humans and no conclusions can be drawn. Some endocrine disruptors may compromise normal ovarian hormone production, both directly through effects on the granulosa or theca cells and indirectly through involvement of the factors necessary for steroidogenesis (12). Changes in the concentrations of the main sex hormones can lead to different clinical phenotypes, depending on the dose and exposure time. In mouse models, prenatal exposure to low doses of BPA reduces testosterone, oestradiol and aromatase production, while at high doses the effects are diametrically opposed (68-69). The "partial agonism/antagonism" mechanism is responsible for this "U" response to some substances: at low concentrations they compete with the endogenous ligand, which is more efficient at signal translation, while at high concentrations they saturate the receptor, in any case causing it to be activated.

Data from human studies are less conclusive. However, in vitro studies have demonstrated with reasonable certainty that phthalates and pesticides can also affect normal hormone production (70-71). In any case, documenting these effects requires highly accurate measurement of hormone levels and the ability to assess the steroidogenic profile as a whole, such as through use of liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Fertility, pregnancy and menopause

As described, the effects of exposure to endocrine disruptors on steroidogenesis and gametogenesis result in clinical phenotypes of varying severity. Fertility may be compromised following hormone imbalances or morphofunctional follicular disorders; however, other mechanisms also seem to be involved in the impairment of female fertility. For example, urine concentrations of BPA have been associated with a greater likelihood of assisted reproduction technique (ART) failure (72), and significantly higher plasma concentrations have been observed in infertile women in comparison with controls (73). Exposure to BPA also seems to be associated with faster depletion

of the ovarian reserve. Data on the possible role of phthalates and pesticides on female fertility are inconclusive, although an association between high concentrations and earlier menopause has also been documented in humans (74).

Endocrine disruptors and immune and autoimmune diseases

Immune system abnormalities such as hypersensitivity, autoimmunity and immunomodulation can affect quality of life. Recent studies demonstrate that numerous chemicals introduced into the environment interfere with the human immune system. The consequences of such interference in developing organisms are not yet fully clear. However, there are some situations in which immune impairment can be recognised as having been caused by chemicals identified as immunotoxic.

Allergies are the most common cause of chronic disease in developed countries, and their incidence is also rising in developing countries. Clinical data and scientific evidence suggest that the incidence of allergy in childhood may be linked to environmental aspects correlated with hypothalamus-pituitary axis (HPA) disorders. Inhibition or activation of the HPA during embryonal development and early childhood could contribute to the development of allergic responses (75).

Autoimmune diseases, which are also rising sharply, are another event potentially induced by EDCs. To date, more than 80 systemic and organ-specific autoimmune diseases have been identified, producing a substantial medical and financial burden. Around 5-7% of the population of developing countries worldwide are affected, and the social cost is constantly rising. This makes these diseases an omnipresent global phenomenon, and their incidence is predicted to rise further over the next decades. The strongest rise has been seen in new cases of type 1 diabetes mellitus. Data from 17

European countries demonstrate a mean increase of 3.9% (1989-2003), and this is expected to double by 2020 with respect to 2005 (76).

It should be remembered that the risk of onset of autoimmune diseases depends on both genetic makeup (intrinsic predisposition to the development of diseases) and the environment. For example, people of African descent living in London are four times as likely to develop systemic lupus erythematosus as those living in sub-Saharan Africa. However, direct evidence for links between environment and disease is weak, mainly due to the intrinsic limitations of environmental epidemiology (78). Furthermore, while there is largescale human exposure to phthalates, studies are often limited by the difficulty of classifying the diseases and by their statistical power.

Many immune disorders are deeply rooted in the endocrine system, as the inappropriate activation or deactivation of endocrine pathways can disturb the equilibrium of the immune response in an aberrant manner: the endocrine and immune systems are in fact intrinsically connected to ensure the simultaneous management of infections, stress and reproduction (79). As discussed above, EDCs have been directly implicated in disorders affecting the metabolism, energy balance, thyroid function and reproduction, as well as in an increased risk of endocrine tumours (80).

There is considerable scientific evidence that endocrine disruptors are involved in the development of autoimmune diseases. Autoimmune thyroid diseases are the most common organ-specific autoimmune diseases, affecting an estimated 5% of the population (81). They are caused by disruption of the interaction between the thyrocytes (the cells that are the functional units of the thyroid) and the immune system (T lymphocytes), culminating in an autoimmune reaction in which the immune system attacks the organ. There is considerable scientific evidence that EDCs can affect the aetiology of these diseases, perhaps by modifying the normal immune-endocrine interaction and forcing it along an autoimmune pattern (5, 82).

EDC and endometriosis

Endometriosis is the most common cause of pelvic pain and infertility in women. Cytokines and growth factors have been indicated as factors involved in its onset (83).

Numerous clinical studies demonstrate a direct correlation between plasma concentrations of phthalates and their esters (plasticisers) and endometriosis (84). Dioxins have also been implicated (85-86). Dioxin-like compounds and PCBs (synthetic mixtures used as paints, resins, plastics, printing inks and glues) have been found in high concentrations in the blood of women with endometriosis (87-88). Accidental exposure to dioxins following the previously mentioned industrial accident in Seveso, Italy resulted in an increased risk of endometriosis (89). However, despite the evidence linking EDCs in the blood to endometriosis, there is little evidence suggesting a mechanism or establishing a causal relationship. The action of these chemicals on the aryl receptor was recently highlighted, producing new lines of research.

References

1. Colao A, Muscogiuri G, Piscitelli P. Environment and Health: Not Only Cancer. Int J Environ Res Public Health. 2016 Jul 19;13(7).
2. Panzica GC, Viglietti-Panzica C, Mura E, Quinn MJ, Lavoie E, Palanza P, Ottinger MA. Effects of xenoestrogens on the differentiation of behaviorally- relevant neural circuits. Front Neuroendocrinol 2007; 28: 179-200
3. Muscogiuri G, Colao A. Phtalates: new cardiovascular health disruptors? Arch Toxicol. 2016 Jun 29. [Epub ahead of print]
4. Gore AC. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. Front Neuroendocrinol. 2008; 29:358-374
5. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009; 30:293-342.
6. AIOM-AIRTUM: I numeri del cancro in Italia 2016. 2016.
7. Davies L, Welch H.G.. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 2014;140:, 317-322.
8. Oh CM, Jung KW, Won YJ, Shin A, Kong HJ, Lee JS. Age-Period-Cohort Analysis of Thyroid Cancer Incidence in Korea. Cancer Res Treat 2015; 47:, 362-369.
9. Howlader N, Krapcho M, Neyman N, Aminou R, Waldron W, et al. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). 2012.
10. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013; 13: 184-199.
11. Gutleb AC, Cambier S, Serchi T. Impact of Endocrine Disruptors on the Thyroid Hormone System. Horm Res Paediatr 2016.
12. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36(6):E1-E150
13. Maqbool F, Mostafalou S, Bahadar H, Abdollahi M. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. Life Sci. 2016; 145:265-73.
14. Duntas LH, Stathatos N. Toxic chemicals and thyroid function: hard facts and lateral thinking. Rev Endocr Metab Disord 2015;16:, 311-318.
15. Guan H, Ji M, Bao R, Yu H, et al. Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer. J Clin Endocrinol Metab 2009; 94:, 1612-1617.
16. Pellegriti G, De Vathaire F, Scollo C, et al. Papillary thyroid cancer incidence in the volcanic area of Sicily. J Natl Cancer Inst 2009; 101: 1575-1583.
17. Marcello MA, Malandrino P, et al. The influence of the environment on the development of thyroid tumors: a new appraisal. Endocr Relat Cancer 2014; 21: T235-254.
18. Leux C, Guenel P.Risk factors of thyroid tumors: role of environmental and occupational exposures to chemical pollutants. Rev Epidemiol Sante Publique 2010; 58: 359-367.
19. Zaidi SS, Bhatnagar VK, Gandhi SJ, et al. Assessment of thyroid function in pesticide formulators. Hum Exp Toxicol 2000; 19, 497-501.
20. Cannavò S, Ferraù F, Ragonese M, Curtò L, Torre ML, Magistri M, Marchese A,Alibrandi A, Trimarchi F. Increased prevalence of acromegaly in a highly polluted area. Eur J Endocrinol. 2010; 163: 509-13.
21. Pesatori AC, Baccarelli A, Consonni D, et al. Aryl hydrocarbon receptor-interacting protein and pituitary adenomas: a population-based study on subjects exposed to dioxin after the Seveso, Italy, accident. Eur J Endocrinol. 2008; 159: 699-703.
22. Denison MS, Pandini A, Nagy SR, Baldwin EP, Bonati L. Ligand binding and activation of the Ah receptor. Chem Biol Interact. 2002; 141: 3-24.
23. Cannavò S, Ragonese M, Puglisi S, et al. Acromegaly Is More Severe in Patients With AHR or AIP Gene Variants Living in Highly Polluted Areas. J Clin Endocrinol Metab. 2016; 101: 1872-1979.
24. Heindel JJ, Newbold R, Schug TT. Endocrine disruptors and obesity. Nat Rev Endocrinol. 2015 ; 11: 653-61.

25. Cheikh Rouhou M, Karelis AD, St-Pierre DH, Lamontagne L. Adverse effects of weight loss: Are persistent organic pollutants a potential culprit? Diabetes Metab. 2016; 42(4):215-23.
26. Migliaccio S, Newbold RR, McLachlan JA, Korach KS. Alterations in estrogen levels during development affects the skeleton: use of an animal model. Environ Health Persp 1995; 103:97-99.
27. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol 2010; 6:363-370.
28. Paoli D, Giannandrea F, Gallo M, Turci R, Cattaruzza MS, Lombardo F, Lenzi A, Gandini L. Exposure to polychlorinated biphenyls and hexachlorobenzene, semen quality and testicular cancer risk. J Endocrinol Invest. 2015; 38(7):745-52
29. Prusinski L, Al-Hendy A, Yang Q. Developmental exposure to endocrine disrupting chemicals alters the epigenome: Identification of reprogrammed targets. Gynecol Obstet Res. 2016; 3(1):1-6.
30. Grün F, Blumberg B. Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. Rev Endocr Metab Disord. 2007; 8(2):161-71.
31. Migliaccio S, Greco EA, Wannenes F, Donini LM, Lenzi A. Adipose, bone and muscle tissues as new endocrine organs: role of reciprocal regulation for osteoporosis and obesity development. Horm Mol Biol Clin Investig. 2014;17(1):39-51.
32. Lubrano C, Genovesi G, Specchia P, Costantini D, Mariani S, Petrangeli E, Lenzi A, Gnessi L. Obesity and metabolic comorbidities: environmental diseases? Oxid Med Cell Longev. 2013;2013:640673.
33. Nelson JW, Hatch EE, Webster TF. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect. 2010; 118(2):197-202.
34. Kelishadi R, Poursafa P, Jamshidi F. Role of environmental chemicals in obesity: a systematic review on the current evidence. J Environ Public Health 2013;2013:896789
35. Regnier SM, El-Hashani E, Kamau W, Zhang X, Massad NL, Sargis RM. Tributyltin differentially promotes development of a phenotypically distinct adipocyte. Obesity (Silver Spring). 2015;23(9):1864-71.
36. Zuo Z, Chen S, Wu T Tributyltin causes obesity and hepatic steatosis in male mice. Environ Toxicol 2011; 26:79-85.
37. Harada S, Hiromori Y, Nakamura S, et al. Structural basis for PPARgamma transactivation by endocrine-disrupting organotin compounds. Sci Rep 2015; 5:8520.
38. Janesick AS, Blumberg B. Obesogens: an emerging threat to public health. Am J Obstet Gynecol. 2016; 214(5):559-65.
39. Masuno H, Iwanami J, Kidani T, Sakayama T, Honda K Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway, Toxicol. Sci. 2005; 84 319–327.
40. Valentino R, D'Esposito V, Passaretti F, Liotti A, Cabaro S, Longo M, Perruolo G, Oriente F, Beguinot F, Formisano P. Bisphenol-A impairs insulin action and up-regulates inflammatory pathways in human subcutaneous adipocytes and 3T3-L1 cells. PLoS One. 2013; 8(12):e82099.
41. Ariemma F, D'Esposito V, Liguoro D, Oriente F, Cabaro S, Liotti A, Cimmino I, Longo M, Beguinot F, Formisano P, Valentino R. Low-Dose Bisphenol-A Impairs Adipogenesis and Generates Dysfunctional 3T3-L1 Adipocytes. PLoS One. 2016;11(3):e0150762.
42. Tarantino G, Valentino R, Di Somma C, D'Esposito V, Passaretti F, Pizza G, Brancato V, Orio F, Formisano P, Colao A, Savastano S. Bisphenol A in polycystic ovary syndrome and its association with liver-spleen axis. Clin Endocrinol (Oxf). 2013 Mar;78(3):447-53.
43. Provvisiero DP, Pivonello C, Muscogiuri G, Negri M, de Angelis C, Simeoli C, Pivonello R, Colao A. Influence of Bisphenol A on Type 2 Diabetes Mellitus. Int J Environ Res Public Health. 2016 Oct 6;13(10).
44. Chamorro-García R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. Environ Health Perspect. 2013;121(3):359-66.

45. Biemann R, Navarrete Santos A, Navarrete Santos A, Riemann D, Knelangen J, Blüher M, Koch H, Fischer B. Endocrine disrupting chemicals affect the adipogenic differentiation of mesenchymal stem cells in distinct ontogenetic windows. Biochem Biophys Res Commun. 2012;417(2):747-52.
46. Phillips KP, Foster WG. Key developments in endocrine disrupter research and human health. J Toxicol Environ Health B Crit Rev. 2008;11(3-4):322-344.
47. Crinnion WJ. Maternal levels of xenobiotics that affect fetal development and childhood health. Altern Med Rev. 2009;14(3):212-222.
48. Unuvar T, Buyukgebiz A. Fetal and neonatal endocrine disruptors. J Clin Res Pediatr Endocrinol. 2012;4(2):51-60.
49. Abaci A, Demir K, Bober E, Buyukgebiz A. Endocrine disrupters - with special emphasis on sexual development. Pediatr Endocrinol Rev. 2009;6(4):464-475.
50. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972-978.
51. Bonde JP, Flachs EM, Rimborg S, et al. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum Reprod Update. 2016.
52. Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: a meta-analysis. J Pediatr Urol. 2009;5(1):17-24.
53. Nassar N, Abeywardana P, Barker A, Bower C. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med. 2010;67(9):585-589.
54. Hass U, Scholze M, Christiansen S, et al. Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. Environ Health Perspect. 2007;115 Suppl 1:122-128.
55. Hosie S, Loff S, Witt K, Niessen K, Waag KL. Is there a correlation between organochlorine compounds and undescended testes? Eur J Pediatr Surg. 2000;10(5):304-309.
56. Krysiak-Baltyn K, Toppari J, Skakkebaek NE, et al. Association between chemical pattern in breast milk and congenital cryptorchidism: modelling of complex human exposures. Int J Androl. 2012;35(3):294-302.
57. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res. 2008;108(2):177-184.
58. Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. Int J Androl. 2006;29(1):228-234.
59. Vested A, Giwercman A, Bonde JP, Toft G. Persistent organic pollutants and male reproductive health. Asian J Androl. 2014;16(1):71-80.
60. Specht IO, Hougaard KS, Spano M, et al. Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances - a study of spouses of pregnant women in three geographical regions. Reprod Toxicol. 2012;33(4):577-583.
61. Mocarelli P, Gerthoux PM, Needham LL, et al. Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ Health Perspect. 2011;119(5):713-718.
62. Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. Lancet. 2000;356(9237):1240-1241.
63. Santangeli S, Maradonna F, Olivotto I, Piccinetti CC, Gioacchini G, Carnevali O. Effects of BPA on female reproductive function: The involvement of epigenetic mechanism. Gen Comp Endocrinol. 2016.
64. Li Y, Zhang W, Liu J, et al. Prepubertal bisphenol A exposure interferes with ovarian follicle development and its relevant gene expression. Reprod Toxicol. 2014;44:33-40.
65. Craig ZR, Hannon PR, Wang W, Ziv-Gal A, Flaws JA. Di-n-butyl phthalate disrupts the expression of genes involved in cell cycle and apoptotic pathways in mouse ovarian antral follicles. Biol Reprod. 2013;88(1):23.
66. Jefferson WN. Adult ovarian function can be affected by high levels of soy. J Nutr. 2010;140(12):2322S-2325S.

67. Lee H, Lim S, Yun S, Yoon A, Park G, Yang H. Tributyltin increases the expression of apoptosis- and adipogenesis-related genes in rat ovaries. Clin Exp Reprod Med. 2012;39(1):15-21.
68. Delclos KB, Camacho L, Lewis SM, et al. Toxicity evaluation of bisphenol A administered by gavage to Sprague Dawley rats from gestation day 6 through postnatal day 90. Toxicol Sci. 2014;139(1):174-197.
69. Lee SG, Kim JY, Chung JY, et al. Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17beta-estradiol synthesis via downregulation of aromatase in rat ovary. Environ Health Perspect. 2013;121(6):663-669.
70. Luderer U, Kesner JS, Fuller JM, et al. Effects of gestational and lactational exposure to heptachlor epoxide on age at puberty and reproductive function in men and women. Environ Res. 2013;121:84-94.
71. Hart R, Doherty DA, Frederiksen H, et al. The influence of antenatal exposure to phthalates on subsequent female reproductive development in adolescence: a pilot study. Reproduction. 2014;147(4):379-390.
72. Ehrlich S, Williams PL, Missmer SA, et al. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. Environ Health Perspect. 2012;120(7):978-983.
73. Caserta D, Bordi G, Ciardo F, et al. The influence of endocrine disruptors in a selected population of infertile women. Gynecol Endocrinol. 2013;29(5):444-447.
74. Grindler NM, Allsworth JE, Macones GA, Kannan K, Roehl KA, Cooper AR. Persistent organic pollutants and early menopause in U.S. women. PLoS One. 2015;10(1):e0116057.
75. Buske-Kirschbaum A. Cortisol responses to stress in allergic children: interaction with the immune response. Neuroimmunomodulation. 2009;16(5):325-332.
76. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G, Group ES. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet. 2009;373(9680):2027-2033.
77. Ramos RG, Olden K. Gene-environment interactions in the development of complex disease phenotypes. Int J Environ Res Public Health. 2008;5(1):4-11.
78. Schmidt CW. Questions persist: environmental factors in autoimmune disease. Environ Health Perspect. 2011;119(6):A249-253.
79. Khardori R, Adamski A, Khardori N. Infection, immunity, and hormones/endocrine interactions. Infect Dis Clin North Am. 2007;21(3):601-615, vii.
80. Walker DM, Gore AC. Transgenerational neuroendocrine disruption of reproduction. Nat Rev Endocrinol. 2011;7(4):197-207.
81. Klecha AJ, Barreiro Arcos ML, Frick L, Genaro AM, Cremaschi G. Immune-endocrine interactions in autoimmune thyroid diseases. Neuroimmunomodulation. 2008;15(1):68-75.
82. Zoeller TR. Environmental chemicals targeting thyroid. Hormones (Athens). 2010;9(1):28-40.
83. Seli E, Arici A. Endometriosis: interaction of immune and endocrine systems. Semin Reprod Med. 2003;21(2):135-144.
84. Cobellis L, Latini G, De Felice C, et al. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. Hum Reprod. 2003;18(7):1512-1515.
85. Mayani A, Barel S, Soback S, Almagor M. Dioxin concentrations in women with endometriosis. Hum Reprod. 1997;12(2):373-375.
86. Koninckx PR, Braet P, Kennedy SH, Barlow DH. Dioxin pollution and endometriosis in Belgium. Hum Reprod. 1994;9(6):1001-1002.
87. Porpora MG, Ingelido AM, di Domenico A, et al. Increased levels of polychlorobiphenyls in Italian women with endometriosis. Chemosphere. 2006;63(8):1361-1367.
88. Heilier JF, Nackers F, Verougstraete V, Tonglet R, Lison D, Donnez J. Increased dioxin-like compounds in the serum of women with peritoneal endometriosis and deep endometriotic (adenomyotic) nodules. Fertil Steril. 2005;84(2):305-312.

89. Eskenazi B, Mocarelli P, Warner M, et al. Seveso Women's Health Study: a study of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on reproductive health. Chemosphere. 2000;40(9-11):1247-1253.