Endocrine Disruptors and Human Health

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Abstract: Endocrine-disrupting chemicals (EDCs) are a group of diversely natural compounds or synthetic chemicals that can interfere with the programming of normal endocrine-signalling pathways during pre- and neonatal life, thus leading to adverse consequences later in life. In addition, early life exposure to EDCs may alter gene expression and consequently transmit these effects to future generations.

Keywords: Endocrine-disruptors, environment, endocrine system, phthalates, pregnancy, neonate, fetal.

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are a large and increasing group of diversely natural compounds or synthetic chemicals present in the environment that include persistent halogenated pollutants, such as polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and metabolites, industrial compounds, such as bisphenol A (BPA), alkylphenols and phthalate acid esters, as well as pharmaceuticals, pesticides, such as chlorpyrifos, fungicides including vinclozalin and phytoestrogens.

Man-made EDCs range across all continents and oceans. EDCs, which are typically present as complex mixtures and not as single substances, may mimic, block or modulate the synthesis, release, transport, binding, metabolism and/or elimination of natural endogenous hormones in wild animals and humans [1]. In particular, EDC may interfere with hormonal signalling systems and alter feedback loops in the brain, pituitary, gonads, thyroid, and other components of the endocrine system.

Growing evidence shows that EDC may also modulate the activity/expression of steroidogenic enzymes and steroidogenic pathways [2-5].

In addition, EDC can also promote activation of metabolic sensors, such as the peroxisome proliferator-activated receptors (PPARs) [6]. As a consequence, there is an increasing concern worldwide on the potential adverse effects of ED on human health, although their impact on human beings' health is not yet clear.

However, endocrine signalling pathways play an important role during prenatal differentiation; thus, developing organisms may be particularly sensitive to ED effects. In fact, scientific evidence indicate that exposure to ED during critical periods of development can induce permanent changes in several organs, including molecular alterations, although the consequences of this disruption may not appear until later [7-11]. The mechanisms by which ED exert their action remain largely unclear; however, many ways have been identified by which ED can affect signal transduction systems [12].

Early life exposures to EDCs may alter gene expression *via* non-genomic, epigenetic mechanisms, including DNA methylation and histone acetylation, thus interfering with the germ-line. By contaminating the environment with ED human race might be permanently affecting the health of subsequent generations [13-15]. Within the broad ED topic we have focussed on specific issues, selected since they are highly relevant to the up-to-date assessment of potential human health risks from ED exposure.

ED IN THE FOOD CHAIN: HOW THEY INTERACT WITH NATURAL COMPOUNDS?

Diet is a significant source of exposure to ED for the general population, as well as a source of concern for consumers' health. One major issue is the "cocktail" effect: one cannot rule out additivity of different ED present in whole diet at low level, but hitting the same targets, e.g. nuclear receptors [16]. Furthermore, it is not just the daily dose that matters. Many ED can bioaccumulate in lipid fraction of tissues, originating a mixture "body burden" of contaminants of different origin that can include dioxins, polychlorinated biphenyls, chlorinated pesticides and their metabolites, as well as brominated flame retardants [17]. Other compounds may also concentrate in food chains, thus adding to the overall ED burden, e.g., organotins [18]. However, the modern conception of food toxicology cannot consider diet just as an exposure source of external harmful substances. Contaminants such as ED may interact with the same metabolic pathways as natural food components such as polyunsatu-

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rated fatty acids, trace elements, vitamins and other bioactive substances (e.g. polyphenols) that cannot be considered nutrients as there is no recognized deficiency [19]. Dietary habits are related to socioeconomic status, cultural and religious factors, individual choices (e.g. vegetarianism/veganism); and dietary habits themselves may have the most important impact on the intake of both nutrients and contaminants. For instance, greater exposure to persistent ED is associated with the high consumption of fatty foods of animal origin [20, 21]. Thus, for specific food commodities a balanced evaluation is needed about contaminant-associated risks and nutritional benefits. A relevant example is represented by salmonids and other seafood, a useful source of nutrients such as polyunsaturated fatty acids as well as a major source of ED and other bioaccumulating contaminants, such as methylmercury. Evidence might justify recommendations to increase as well as to reduce fish consumption, quite an uneasy situation for risk managers: decreasing fish consumption (and its nutritional benefits) may not be necessary in Europe, but monitoring of contaminants in edible fish should be continued, as well as the development of novel aquaculture feeds, less liable to contamination [22].

Most important, effects of contaminants and natural food components may interact on the same pathways and targets. The outcomes of interactions may be complex, depending on dose and targets; e.g., phytoestrogens can protect against some hormone-dependent cancers, as well as postmenopausal osteoporosis, but may also interfere with receptor-mediated signal transduction (e.g. by inhibiting protein kinase) and DNA replication [23]. Up to date, scientific data available on interactions between xenobiotics and "natural" substances in food are still limited; below, some relevant examples are provided

Iodine and ED

Iodine is the main determinant of thyroid development and function; seafood and milk are the main dietary sources. Subclinical iodine deficiency is still a common problem in many areas, including Europe [24]; thyroid is also increasingly recognized as a major target for ED, including newly recognized ones, such as organpophopsphorus insecticides [25]. Yet, only a few papers target low iodine status in relation to susceptibility to xenobiotics. Somewhat unexpectedly phthalates, the widespread plasticizers known mainly as antiandrogens, can modulate basal iodide uptake mediated by the sodium/iodide symporter in thyroid follicular cells *in vitro*: the effect was not shared by all phthalates and was independent from cytotoxicity [26]. Many phytoestrogens may interfere with iodination of thyroid hormones. Some (e.g., naringenin, and quercetin, which contain a resorcinol moiety) are direct and potent inhibitors of thyroid peroxidase, others (myricetin, naringin) show noncompetitive inhibition of tyrosine iodination with respect to iodine ion, whereas biochanin A may act as an alternate substrate for iodination [27]. A Czech biomonitoring study in children also indicated an adverse effect of genistein on thyroid function [28]. The drinking-water contaminant perchlorate inhibits thyroidal iodide uptake; however, iodine-deficient female rats were more resistent to the inhibition of iodine absorption from perchlorate exposure than normal rats [29]. Thus, the interaction between iodine and some thyroid-targeting ED may be less straightforward than expected.

Phytoestrogens and the "xeno"ED

Due to their pleomorphic biological effects, phytoestrogens are a sort of "natural ED", whose overall dietary intake of phytoestrogens may be significant also in Europe [23, 30, 31]. Flavonoids (daidzein, genistein, quercetin, and luteolin) can at least partly antagonize the proliferation-stimulating activity of synthetic estrogenic ED in estrogen-dependent MCF-7 human breast cancer cells: thee ED included anionic detergent by-products alkylphenols, plastic additive bisphenol A, and the PCB 4-dihydroxybiphenyl [32, 33]. These findings suggest that phytoestrogens can compete with estrogenic ED on shared biological targets, thus exerting a protective action . In other models no interaction was observed: genistein did not modulate the effects on human astroglial cells by two persistent ED, the polybrominated flame retardant PBDE-99 and the PCB mixture Aroclor 1254 [34]. As it is sometimes the case, *in vivo* studies provide a more complex picture. Genistein and the estrogenic chlorinated insecticide methoxychlor had an additive impact on both immune function and immune functional development in rats; the developing thymus appeared as a sensitive target of combined exposure [35]. In estrogen reporter (ERE-tK-Luciferase) male mice genistein modulated the actions of both estradiol and persistent ED in liver and testis with tissue-specific features: the antiestrogenic action of beta-hexachlorocyclohexane in the testis and o,p'-DDT in the liver was antagonized, whereas genistein had an additive effect with the ER agonist p,p'-DDT in the liver [36]. Two predefined mixtures of phytoestrogens and synthesis ED were tested in the uterotrophic assay on prepubertal rats: the composition of each mixture (what chemicals and to what amount) was based on human exposure data. The phytoestrogen mixture did elicit an uterotrophic response, whereas the synthetic one has no effect itself nor an additive effect with phytoestrogens, possibly because of exposure levels too low [37]. The combined exposure to estrogenic and antiandrogenic ED is suggested as a potential risk to male reproductive development. Genistein and the antiandrogenic fungicide vinclozolin, alone or in combination, were investigated concerning the induction of hypospadias in mice: the incidences were 25%,, 42% and 41% for genistein, vinclozolin and combined treatment, respectively, indicating a less than additive effect [38]. On the other hand, genistein, as well as the methyl donor folic acid, both antagonized the DNA hypomethylating effect of bisphenol A in mouse embryos [39]. The available data indicate that interactions between phytoestrogens and ED can be important, but cannot simply explained in terms of additivity or antagonism; indeed, additivity and antagonism may vary, depending on the chemicals, endpoints and lifestages.

ED and Vitamin A Pathways

Retinoic acid is the internal form of vitamin A interacting with the nuclear receptors RAR and RXR, whose natural ligands are all-trans-retinoic acid and 9-cis-retinoic acid, respectively. Retinoic acid pathways cross-talk with those of the aryl hydrocarbon receptor (AhR), the direct cell target for dioxins and dioxin-like compounds [40]. Dioxins are potent

inducers of cytochrome P450 (CYP) 1A1, that in its turn may enhance the dioxin effects; the concurrent supplementation of vitamin A inhibits CYP1A1 activity in dioxin-exposed mice, reducing liver damage as well as CYP1A1 and AhR mRNA expression [41]. Mice lacking retinoid binding proteins were especially responsive to dioxin-induced liver retinoid depletion, intracellular retinoid binding protein I being the main factor. RAR- and RXR-knockout mice were essentially sensitive as wild-type mice, with the exception of RXRbeta-/- mice which showed no decrease in hepatic Vitamin A concentration; this suggest a possible role of RXRbeta in dioxin-induced retinoid disruption [42]. Retinoid storage and metabolism were also disrupted in female rats of two strains with different dioxin sensitivity (Long-Evans and Han/Wistar) [43]. Comparison of dioxin effects on liver retinyl palmitate in AhR+/- and AhR-/- mice support disruption of retinoid homeostasis as a primary AhR- mediated mode of action of dioxin-like chemicals [44]. Retinoid pathways can be a critical target also for polybrominated diphenyl ethers: in rats treated orally with pentaBDE-71, decrease of hepatic apolar retinoids was the most sensitive effect, together with reduced thyroid hormone [45]. These studies might also hint to vitamin A deficiency as a susceptibility factor towards some persistent ED.

Although the portfolio of scientific evidence is still quite limited, several other examples can be retrieved from the Endocrine disrupting chemicals – Diet Interaction Database - EDID, the only dedicated database available on ED-nutrient interactions [19]. One further instance is the general protective action elicited by "antioxidant" vitamins C and E towards the effects of several EDs, including dioxin-like polychlorinated byphenyls (PCB) and phthalates; indeed, several ED-related modes of action seem to eventually lead to increased oxidative stress [46]. Overall, new evidence on interactions between ED and natural food components may disclose new insights on food-related factors modulating vulnerability as well as on nutrient intake as support to risk prevention and/or risk reduction strategies.

EXPOSURE TO EDCS AND IMPACT ON THE FETO-PLACENTAL UNIT

Maternal exposure to EDCs has been demonstrated to be a significant reason for increases in adverse pregnancy and fetal outcomes. The placenta protects and nourishes the fetus by regulating nutrient and xenobiotic homeostasis between the maternal and fetal compartments. As discussed below, xenobiotics that can affect this placental homeostatic control may lead to abnormal fetal development by altering fetal exposure to toxic compounds and/or nutrient homeostasis [65, 66, 69]. An important aspect of this review is to highlight some areas in which EDCs have drawn considerable attention due to the many potential fetotoxic effects, which may be caused upon *in utero* exposure. For example, recent evidence suggests a link between EDC exposure and the fetal origins of neurological impairment that cannot be ignored even though their mechanistic basis is not well understood [47-51]. EDCs are hypothesized to induce functional and/or structural changes in specific neuroendocrine pathway(s), effects being largely dependent upon the phase (gestational time) and level of exposure [52]. In addition, the potential for additive or synergistic effects of low dose combinations of EDCs are not well established and require considerable investigation. [53]. Moreover, the role of other factors including diet, exercise and genetics has not been well characterized, adding to the difficulty in delineating the role of EDCs on neurodevelopment. Finally, the pharmacokinetic and pharmacodynamic relationships for EDCs differ and there exists a potential for placental and fetal accumulation not accurately measured in maternal plasma [52, 54]. For example, a recent study revealed that when both newborns and adults are exposed to the same bisphenol A (BPA) levels, newborns retain up to 3 times more than adults [55].

BPA is an EDC due to its ability to interact with estrogen receptor (ERα and β) isoforms, androgen receptors (AR), and possessing a high affinity for the estrogen related receptor α (ERRα) during mammalian brain development [56]. BPA eluted from polycarbonate drinking bottles was demonstrated to exert an estrogenic like neurotoxic effect in developing cerebellar neurons [49]. BPA has also been demonstrated to alter fetal neurodevelopment through thyroid hormone (TH) pathways, as recently in a TH-dependent dendritic Purkinje cell development in a murine cerebellar culture assay [51, 57].

BPA is metabolized into BPA glucuronide, which is hormonally inactive, and excreted *via* the urine with a half-life below 6 hours [58]. Despite the rapid metabolism, the U.S. CDC have found BPA levels in 95% of all human urine samples tested, suggesting broad and continuous BPA exposure [59, 60]. Chronic exposure to low levels of BPA may still cause developmental toxicity due to the significant potential for bioaccumulation in the human placenta and fetus [61]. For example, human BPA levels in the placenta and amniotic fluid were 5 folds higher at weeks 15 to 18 compared to maternal serum [62]. Furthermore, fetal and perinatal exposure to BPA has been linked to several neurodevelopmental changes and disorders including autism and the related autism spectrum of disorders (ASD), schizophrenia, impaired neurotransmission, attention deficit and hyperactivity disorders, and potentially sexual dimorphic related changes in brain structure and function, as recently reviewed by Brown [51].

Phthalates are a ubiquitous class of environmental teratogens capable of exerting their toxic effects through several nuclear hormone receptors including the androgen receptor (AR) antagonism [52, 63], ER agonism [52, 64] and/or *trans*-activation of the peroxisome-proliferator activated receptors (PPAR) α and γ isoforms, either directly or indirectly [65-69]. Phthalates are classified as peroxisome proliferator chemicals due to their effects on peroxisomal lipid metabolism [70]. Moreover, they may also exert their neurotoxic effects through altering zinc metabolism [71, 72] or by altering intracellular Ca2+ concentrations leading to the formation of reactive oxygen species potentially through a protein kinase C mediated pathway [73]. It has also been suggested that DEHP inhibits membrane Na+-K+ ATPase in the rat brain, a phenomena linked to several neurodegenerative and psychiatric disorders [74].

Phthalate reproductive toxicology research [63, 65-69] has been largely focused on di-(2-ethylhexyl)-phthalate

(DEHP), an industrial plasticizer that is ubiquitously dispersed in the environment. Human DEHP exposure most likely begins in the mother's womb, where DEHP has been demonstrated to readily cross the placenta and accumulate in the fetus [67, 68]. DEHP mediated direct or indirect PPAR effects on placental essential fatty acid (EFA) homeostasis have also been of interest [64-69]. The fetus requires maternal dietary intake and placental transfer of EFAs to guide proper pregnancy outcomes and fetal development, e.g. neurodevelopment [75-78]. The placenta plays a fetoprotective role by accumulating EFAs from maternal circulation and directionally transporting them into fetal compartment [79, 80]. With regards to proper neurodevelopment, EFAs including docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are known to play critical roles in myelogenesis and serve as essential components in neurogenesis, thus making the fetoprotective role of the placenta essential for neurodevelopment [76-80]. EFA imbalances have been linked to several neurological disorders including autism and ASDs, bipolar disorder, and schizophrenia, suggesting that a proper EFA supply is required to protect the CNS development [81, 82].

PPARα and γ regulate the expression of several fatty acid transport conferring proteins and metabolizing enzymes that maintain essential fatty acid (EFA) homeostasis and can be *trans*-activated by DEHP and its metabolites, mono-(2-ethylhexyl)-phthalate (MEHP) and 2-ethylhexanoic acid (EHA) [65, 66, 83]. Recent studies revealed that DEHP and its metabolites MEHP and EHA, can significantly increase the expression of EFA homeostasis proteins, EFA and lipid accumulation in the lipid metabolome of HRP-1 *in vitro* rat placental cell line [65, 84]. It was also demonstrated that the resulting increase in the expression of fatty acid transport-conferring proteins in these cells also led to a significant increase in fatty acid and lipid accumulation in the cells [85]. DEHP exposure has been revealed to alter the expression of EFA homeostasis proteins in the *in vivo* rat placenta [66]. In this study, radiolabeled AA and DHA where administered to rat dams at gestational day (GD) 20 and the maternal, placental and fetal disposition of the labeled EFAs were assessed [66]. AA was significantly reduced in the maternal and fetal plasma, yet increased significantly in the placenta upon DEHP exposure. DHA levels significantly increased in the maternal plasma and decreased in the fetal plasma upon DEHP exposure contrasted to the control vehicle. DEHP exposure also elicited a statistically significant decrease of both AA and DHA in the developing fetal brain. Lipomic analysis also revealed that DEHP reduced fetal pup brain accumulation of several critical fatty acid and lipid classes including a significant decrease in sphingomyelin (SM) of 54% [69]. DEHP exposure elicited a significant reduction of DHA in five lipid fractions (namely, cholesterol ester (CE), diacylglyceride, phosphatidylserine, lysophasphatidyl choline (LYPC) and SM), whereas AA was significantly decreased CE and LYPC. SM and DHA levels are critical for proper brain development including neurogenesis and neuronal differentiation [76-78]. Moreover, the brain weight and active neuronogenesis rapidly increases from GD15 to term in the rat fetus [79]. These results suggest that DEHP may adversely affect on fetal neurodevelopment.

Recently, it has been found that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can reduce n-3 and n-6 EFAs in contrast to the control when administered to the cynomolgus macaque at GD15 or 20 and the brains were isolated at GD24-26 [85]. Although the mechanism of action was not defined, improper neural tube closing and other neurodevelopmental aberrations were observed and attributed to the improper EFA balance [86-89]. Interestingly, TCDD also has an estrogenic response, acting through ERs, potentially interacting with the aryl hydrocarbon receptor (AHR) in rats [90, 91].

In summary, several EDCs have been demonstrated to exert their teratogenic effects on fetal neurodevelopment. Considerable attention is necessary to elucidate the mechanisms by which individual or multiple combinations of EDCs can elicit fetal neurotoxicity. The extent to which the animal data may be extrapolated to predict a human response will also need to be determined.

ED EXPOSURE AND PAEDIATRIC ENDOCRINE DISORDERS

Normal human sex differentiation, growth and puberty are critically dependent upon hormonal actions, opening up for targeting by EDCs. Recent epidemiological studies demonstrate increasing incidences of related developmental disorders in children originating in embryonic and fetal life [92]. Environmental influences may play an important role in the pathogenesis of such disorders although the factors involved remain to be characterized [7,8].

Disorders of Sex Development (DSD)

It has been hypothesized that certain defined disorders of male sex differentiation may be linked by common pathogenic mechanisms [92]. Cryptorchidism, hypospadia, testicular cancer and poor semen quality are all proposed to be components of this “syndrome”. It has been suggested that exposure to environmental estrogens or anti-androgens may alter the fetal hormonal balance, or compete directly with the androgen receptor, causing undermasculinization of male fetuses. A severe outcome of such action would potentially result in DSD with an intersex phenotype at birth. Less severe disruption may cause maldescended testes or hypospadia. More recently, epigenetic alterations with transgenerational influences have been implicated in the effects of EDCs on reproductive functions [93].

Cryptorchidism

Cryptorchidism is difficult to study due to poor reporting but certain investigations point to an increased incidence in some countries during several decades [94]. Model substances, particularly antiandrogens, have been associated with cryptorchidism in experimental animals [94]. Prenatal exposure to pesticides (as indicated from concentrations in breast milk) has been linked with an increased risk of cryptorchidism [95]. Higher organochlorine concentrations were found in fat samples from cryptorchid boys when compared with control samples [96] but other studies have failed to demonstrate similar correlations [97]. Although data is accumulating, at the present time no single EDC can be convincingly blamed for causing cryptorchidism.

Hypospadia

Hypospadia is underreported to malformation registries, mainly since the most common mild cases are not reported. Still there are reports from reliable sources indicating an increased incidence in European countries. Large regional variation seems to exist [98, 99]. Similarly to cryptorchidism, exposure studies investigating links to EDCs have come to opposing results and it is fair to say that at the present stage of knowledge the data are inconclusive, mainly due to low power and poor disease classification of most published studies.

Puberty

The onset and tempo of puberty are under endocrine control and there is evidence to indicate that EDCs may affect pubertal development [100]. The proposed adverse effects of EDCs are most often related to premature thelarche in girls. Studies in the US have demonstrated a recent trend to an earlier start of puberty in girls, particularly in certain ethnic groups, with a direct correlation to obesity, as assessed as Body Mass Index (BMI). Exposure to EDCs has been proposed to play a role in this novel trend [101]. One group recently found a similar development in Denmark but failed to show a correlation with BMI and levels of reproductive hormones [102]. This indicates an influence by environmental factors, the natures of which are yet to be determined.

Thyroid

EDCs may exert unwanted actions on thyroid function [103]. Links between exposure to PCBs and increased thyroid stimulating hormone have been observed by some [104, 105], but not other authors [106 107]. PCB has been proposed to exert goiterogenic actions [108], and pentachlorophenol indicated to suppress thyroid hormone levels in newborns [109]. Given the critical role of the thyroid for fetal and infant neurodevelopment, such action may be associated with adverse neurodevelopmental consequences in children.

Adrenal

EDCs are known to exert harmful actions in the adrenal cortex. DDT metabolites are well known inhibitors of adrenal function [110], exerting direct cytotoxicity to adrenocortical cells. Certain EDCs may have a negative impact on adrenal steroidogenesis. The phytoestrogen resveratrol was found to suppress glucocorticoid production by inhibiting 21-hydroxylase [5]. This may impair glucocorticoid driven stress responses, which is a crucial component of the host-defense system against infection and injury.

ED AS POSSIBLE RISK FACTORS IN ENDOCRINE AUTOIMMUNITY

Autoimmune endocrine disorders are diseases of the endocrine glands caused by an impaired response of the immune system, which fails to recognize self-antigens and reacts against them [111]. Most autoimmune endocrine disorders target single organs, but often patients have multiple autoimmune endocrine disorders or autoimmune polyglandular syndromes, where, two or more endocrine glands are involved by the autoimmune process [112]. Autoimmune endocrine disorders include: type 1 diabetes mellitus, Hashimoto's thyroiditis, Graves' disease, Addison's disease, autoimmune hypophysitis, autoimmune oophritis, autoimmune hypoparathyroidism, testicular insufficiency, and premature ovarian failure.

Autoimmune diseases are usually the result of an interplay between genetic and acquired factors; the latter including also environmental influences [112]. Autoimmune endocrine diseases often present a sexual dimorphism, which suggests a potential role of sexual hormones on the immune system and therefore their contribution to autoimmune disorders [113].

Several studies have reported that sex steroid hormones can modulate the immune system [114]. Although, it is important to bear in mind that the response to sex hormones can vary among individuals, in relation to age, genetic background, duration and level of exposure; in general estrogens and androgens appear to have opposite effects on the immune system [115]. In particular, estrogen treatment in mice has been associated with reduced number of lymphocytes in several organs as well as with a deregulation in the balance between T and B lymphocytes [115]. The most common finding has been an estrogen-related induction of B-cell hyperactivity and T-cell hypoactivity [115]. This enhanced immunoreactivity in females has been defined as a 'double-edged sword', which on the one hand protects against infections, whereas on the other hand increases the risk of developing autoimmune diseases [116].

There is growing evidence suggesting that EDCs can affect the immune system, promoting the development of autoimmune diseases [117]. An example is given by environmental estrogens, which could exert the same effect than endogenous estrogens on the immune system [113]. EDCs could act on the immune system through different mechanisms [118]: 1) inhibiting the processes involved in establishing tolerance, with generation of autoreactive cells; 2) modifying gene expression in cells involved in the immune response, permitting lymphocytes to respond to signals normally insufficient to initiate a response or permitting the antigen-presenting cells to abnormally stimulate a response; 3) modifying self-molecules such that they are recognized by the immune system as foreign.

Yurino *et al.* [119] have demonstrated that EDCs, such as diethylstilbestrol and bisphenol-A (BPA), can stimulate autoantibody production by B1 cells both *in vitro and in vivo*. The majority of data related to ED and autoimmunity are on animal models of lupus [114]. It has been shown that prolactin or estrogen levels accelerate disease activity in lupus-prone mice or can induce a lupus-like syndrome in normal mice. Scant data are available on the effect of ED specifically on endocrine autoimmune diseases [120] and therefore, this area needs further explorations.

BIOLOGICAL MONITORING OF ENDOCRINE DISRUPTORS AND ASSESSMENT OF HEALTH RISKS: THE CASE OF PHTHALATES

Over the years the need for risk assessments has increased because of the potential health risks related to exposure to EDs in everyday life worldwide. Biological monitoring, defined as measuring concentrations of chemicals and

their metabolites in human body fluids, has been shown to represent an accurate, efficient and cost-effective exposure assessment to environmental pollutants even at low levels [121]. By detecting non-invasive biomarkers through appropriate analytical procedures, human bio-monitoring (HBM) takes into account all routes and sources of exposure, thus representing an ideal instrument for risk assessment and risk management [122, 123]. The sensitivity of HBM methods enables the elucidation of the metabolism and modes of action of pollutants in humans. The diesters of benzene-1,2-dicarboxylic (phthalic) acid, commonly known as phthalates, are a family of industrial compounds with a common chemical structure, dialkyl or alkyl/aryl esters of 1,2-benzenedicarboxylic acid [124]. They are high-production-volume synthetic chemicals and the probability of exposure to these chemicals is high because of their use in plastic items and other common consumer products, including personal-care products (e.g., perfumes, lotions, cosmetics), paint, industrial plastics, medical devices and pharmaceuticals; phthalates are primarily used as plasticizers to impart flexibility to an otherwise rigid polyvinylchloride (PVC) [124]. As these plasticizers are not chemically bound to PVC, they elute at a constant rate from plastic products into the environment. Phthalates have been found everywhere, even in infant formulas and breast milk [125, 126].

Recently, food has been observed to be the predominant intake source of DEHP, whilst other sources, such as enteric coatings in medications, considerably contributed to the daily intake of di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) in an adult population [127, 128]. A comparison of the available data resulting from the determination of the target compound in indoor air and house dust as well as emission studies with the results from the HBM studies reveals that only a small portion of intake takes place *via* the air, dust paths and personal care products, such as cosmetics [129, 130].

Phthalates are rapidly metabolized to their monoesters, which can be further transformed into oxidative metabolites, conjugated, and both free and conjugated metabolites excreted in the urine and faeces [131].

Due to their chemical properties exposure to phthalates does not result in bioaccumulation [132].

Urinary secondary metabolites have been shown to represent ideal biomarkers of exposure to phthalates, allowing accurate assessments of human exposure from multiple sources and routes; the use of metabolites also avoids the analytical problems caused by the risk of sample's contamination by the ubiquitous parent phthalates [133-135].

As there may be significant demographic variations in exposure and/or metabolism of phthalates, health-risk assessments for phthalate exposure in humans should consider different potential risk groups [136].

During the last decades, a great deal of scientific and public concern has been raised about the potential health hazards posed by exposures to phthalates, even in environmental concentrations [137-144].

In particular, phthalates with side-chain lengths C4 to C6 are known to adversely affect the differentiation and function of the reproductive system [145-149].

The Center for the Evaluation of Risks to Human Reproduction (CERHR) identified two specific situations as potentially problematic, the exposure of young children to diisononyl phthalate (DINP) through the use of toys or to DEHP from medical devices [150].

To this regard, exposure assessment have observed that the exposure of children to phthalates exceeds that in adults and the tolerable intake of children is frequently exceeded, in some instances up to 20-fold [151,152].

On the other hand, newborns in the Neonatal Intensive Care Unit (NICU) environment represent a population at particularly increased risk for exposure to DEHP, because of their physical conditions, small body size and contemporary exposure to multiple medical devices containing DEHP. To this regard, it has been documented that these newborns can be exposed up to 100 times above the limit values, depending on the intensity of medical care [153, 154]. As a consequence, the U.S.A and European Union promulgated limitations of use for certain phthalates [155]. Although several risk assessments have been finalized for these chemicals during the last decade, in the future there is the need for risk assessments able to cover a high number of exposure situations and a transparent process of collecting data, thus ensuring the safety of workers and consumers [156].

CONCLUSIONS

There is accumulating evidence suggesting that EDCs represent a large and heterogeneous group of chemical compounds that may potentially affect human health, especially if exposures occur at early stages in life, including both prenatal life and early childhood. Effects are especially relevant in relation to the long-term development of the reproductive and nervous systems, as well as for metabolic programming.

Factors co-modulating the risk, besides age and gender, include diet and lifestyle.

As a consequence, in the future risk assessment of human health should include: (i) population-based estimates of environmental total exposure levels from several sources using HBM; (ii) further studies to assess interactions between several different classes of ubiquitous compounds and their combined effect; iii) characterization of appropriate biomarkers of effective dose and susceptibility for major groups of EDCs.

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