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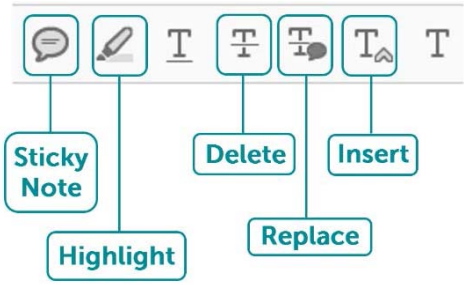

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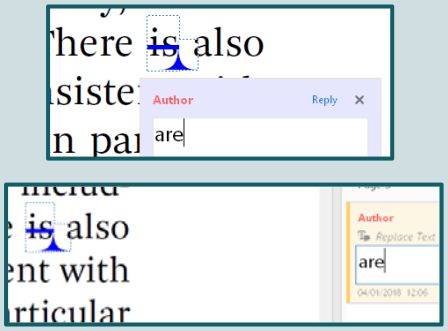

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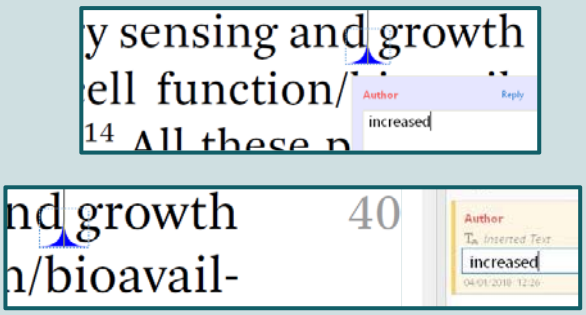

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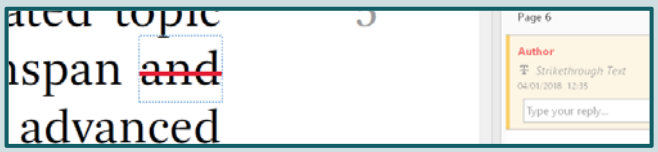

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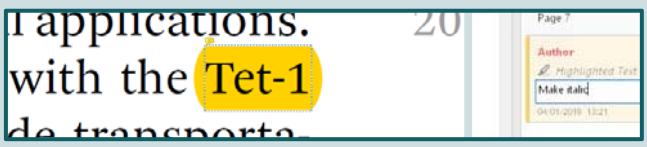

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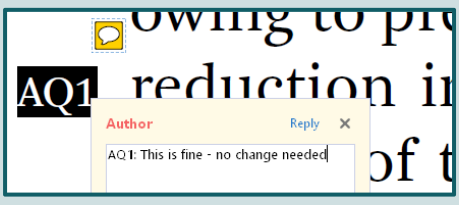

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Human exposure to endocrine disrupting chemicals, particularly at low doses, is ubiquitous, persistent and occurs in complex mixtures with associated health effects that cannot be predicted when analyzing single compounds independently. Concomitant exposure to these compounds, particularly in critical windows of exposure, may therefore result in hazardous health effects in the exposed individuals and in the offspring, potentially associated with a complex 'body burden' of different origins. For the past decades, several epidemiological studies have been performed in an effort to develop biomarkers of effect with the ability to evaluate and potentially predict the risk of disease.

CHAPTER 18

Biomarkers of Effect for EDCs and Indicators to be Used in Epidemiological Studies on Reproductive Health

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18.1 Introduction

Problems regarding endocrine disrupting chemicals (EDCs) exposure derive from the concern caused by scientific data obtained in recent decades that identified several divergent molecular mechanisms mediating effects in human and animal tissues at very low doses and the widespread human exposure to concentrations of EDCs at which adverse effects in animals have been described.[1]

It is acknowledged that, a mixture *per se* does not directly imply a risk to human health, nevertheless there is an urge to implement assessments that examine whether more accurate estimations of risk should be produced by considering the different chemicals present in different mixtures from different

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contexts.[2–4] It is currently accepted that humans are exposed to numerous chemicals through several routes, which can be measured in human tissues, including blood and breast milk, and that these exposures, even at low-doses, are ubiquitous, persistent and occur in complex mixtures with effects that may not be predictable when analyzing single compounds independently.[5]

Another point of debate regarding human exposure to EDCs is that these compounds may not follow the classical monotonic dose responses typically used in toxicological risk assessments but instead may display non-monotonic dose responses (NMDR) curves resultant from multiple mechanisms.[1,6] The Environmental Protection Agency (EPA) determined EDCs' low-dose effects as those observed at concentrations below the levels used for traditional toxicological assessments,[7] however, for the past few years, several studies have demonstrated that extremely low concentrations can induce receptor upregulation with increased responses, whereas higher doses (within the range of typical toxicological assessment) can result in receptor downregulation.[8] Relevantly, hormones and hormone-mimicking chemicals can bind to different receptors (receptor crosstalk),[8] however, EDCs may also act by mechanisms that do not involve direct mediation by classical hormone receptors, such as estrogen receptor alpha or beta (ERα or ERβ). As these compounds may also act on the synthesis or function of enzymes responsible for the synthesis or degradation of hormones and on coregulatory proteins that interact with receptors and, in the case of neurological actions, affect neurotransmitters and their receptors.[8]

Exposures to persistent EDCs, such as polychlorinated biphenyls (PCBs), which have been banned in some countries due to environmental accumulation and potential hazardous health effects, persist in the environment, indoors and in humans.[9] These compounds have been linked with adverse effects in organs such as liver, kidney and thyroid, and endocrine and neurodevelopmental effects due to body burden.[10]

The unintentional exposure of humans to multiple chemicals through multiple routes constitutes the 'mixture' context which has been associated with several outcomes identified through biomarkers of effect, which are the focus of interest in this chapter.

Biomarkers of effect are used to detect and/or measure alterations of the normal morphology, physiology and/or molecular status which can be indicators of an early disease condition derived from an intervention effect or exposure, including exposure to medical products or environmental agents. The serial nature of the measurements focuses attention on changes in the biomarker's value as an indicator of an individual's current or future condition, beneficial or adverse effects of a drug or other intervention, or effects of an exposure over time.[11] The use of biological markers has a variety of contributions to quantitative risk assessment by helping the determination of the forms of dose–time–response relationships; assessment of the biologically effective dose; making interspecies comparison of effective dose, relative potency and effects; resolving the quantitative relationships between human interindividual variability; and identifying subpopulation that are at

enhanced risk.[12,13] Nowadays, most research on biomarkers is concerned with markers which will increase our ability to identify long-term risks due to toxicant exposure, the risk of developing cancer; and identification of early markers of toxicity in the field of environmental health or ecotoxicology. Other potential uses of biomarkers of effect are in monitoring of disease progression and prognosis and as adjuncts to other biomarkers in providing refinements of epidemiology and risk assessments. Finally, biomarkers, offer the opportunity to provide scientific confirmation of proposed exposure–disease pathways *in vivo* in human populations.[12,13] These biomarkers may be particularly useful for demonstrating the biological influence of preceding susceptibility factors, for instance, genetic polymorphisms of xenobiotic-metabolizing enzymes, and it must be stressed that exposure or effect biomarkers are really useful risk assessment tools when the metabolic fate of the compound (toxicokinetics) or the mechanisms of a resultant disease (toxicodynamics) are completely understood.[14,15]

Biomarkers of effect should be linked to biomarkers of exposure and genetic susceptibility. Briefly, a biomarker of exposure is 'an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism'.[16] Exposure biomarkers can reflect bioavailability and be influenced by numerous parameters, such as route of exposure, physiological characteristics of the receptor and chemical characteristics of the xenobiotic. Exposure biomarkers have the advantage of providing an integrated measure of chemical uptake, a consideration that is important in the case of agents that exhibit large route-dependent differences in absorption.[17,18]

A biomarker of susceptibility may be defined as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a chemical.[19,20] Thus any variation in the response of an individual to identical exposures may represent some difference in susceptibility due either to the genetic make-up of the individual or to variables and environmental influences, such as diet or the uptake and absorption of the xenobiotics.[20]

In the future, new and more specific biological markers, predictive of long-term effects should be developed, in order to better understand the insults of substances with endocrine-disrupting activity and fundamentally, to detect the combined effects of mixtures, considering that exposure to a single substance is usually low.

18.2 Biomarkers of Effect

Historically, and in practical terms, these biomarkers have been used most widely and routinely. They can be grouped into different categories: markers which are the result of *pathological damage* – morphologic cellular alterations; and markers which indicate a *metabolic lesion*,[21] measurement of metabolite alterations after a pathological event compared with a normal state or a different pathological process. These metabolic biomarkers can also be used for prognosis and monitoring treatment response.

Biomarkers of effect (also known as biomarkers of response) can be elicited as a result of interaction of the organism with a host of different environmental factors (including chemical, physical and biological agents).[11] A biomarker can be any substance, structure or process that can be monitored in tissues or fluids and that predicts or influences health; or that assesses the incidence or biological behavior of a disease, but is not a measure of disease, disorder or health condition itself.[12,13] Ideally, biomarkers should be accessible (non-invasive), non-destructive, easy and cheap to measure.[14,15]

Biomarkers of effect, which measure processes of genetic damage, are sometimes used to identify exposures. Because of this, classifications may be made in more mechanistic terms, such as reversible (transient) responses (to an exposure or dose) and irreversible (permanent) responses (indicating an early-stage effect).[22] Preferably, a biomarker of effect should reflect early reversible changes in the organism.

One of the criteria for establishing associations between an exposure and disease is biological plausibility. In this context, biomarkers may contribute by illuminating some of the carcinogenic steps linked to a particular risk factor. This is possibly an undervalued area, where biomarkers can make significant contributions to cancer epidemiology.[23]0 For instance, if a particular chemical exposure from ambient air is associated with increased risk, the additional information that exposed individuals have higher levels of DNA damage would add support to the exposure–disease association.[21]

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Biomarkers of effect offer the opportunity to provide scientific confirmation of proposed exposure–disease pathways in human populations, since they can be elicited as a result of interaction of the biological system with the environment.[11,24] The increasing demand for information about health risks derived from exposure to complex mixtures calls for the identification of biomarkers to evaluate not only genotoxic but also other high-concern effects associated with occupational and environmental exposure to chemicals and other potential sources of damage.[23]

18.3 EDCs Environmental Exposure Effects: Epidemiological Studies Biomarkers

Humans are indisputably, ubiquitously and persistently exposed to multiple chemicals found in food, air, drinking water, household and consumer products, thus, the assessment of the cumulative risk to human health or

the environment from multiple chemicals *via* multiple routes or mixture risk assessment (MRA) is imperative.[25]

The World Health Organization (WHO) report on EDC stated: '*. . .there is emerging evidence that many chemicals may act additively and, each at levels without individual effect, could act together to cause health problems*'.[26] Additionally, compounds that have the same effect in the same target organ are assumed to act in an additive way by the European Food Safety Authority (EFSA), even when their chemical structures and molecular mechanisms are different.[27] On the other hand, chemicals with divergent modes of action interact independently with different subsystems,[3] and some compounds (*e.g.* Bisphenol A) can bind to multiple hormone receptors.[28] Likewise, the concept of threshold as it applies to EDCs is equally complicated and must be considered with caution.[29] Thus, EDCs mixture effects extend much beyond the groups of chemicals sharing chemical structures and molecular mechanisms.[30]

Studies focused on the evaluation of mixtures of components with estrogenic, antiandrogen and thyroid-disrupting activities demonstrated the utility of the concept of dose addition in anticipating combination effects, demonstrating that joint effects occur even at levels below doses that cause observable effects,[2] and several biomarkers of effect have been used.

18.4 Critical Windows of Exposure to EDCs and Effects

EDCs' health risks are closely associated with specific life stages with concomitant critical windows of exposure,[27] such as embryonic development and early life, which requires accurate timing of hormone action to promote proper growth and development of tissues and organs. Thus, at prenatal and early life stages of human development, exposure of fetuses and young children to pollutants, such as EDCs, through maternal blood and/or milk can permanently reprogram physiological processes, influencing health and/or reproductive function lather in life and in the progeny.[31] This explains why several epidemiological studies have been dedicated to these periods of life.

18.4.1 Embryonic Development

For the past decades several studies have focused on EDCs effects in sex hormone-responsive organs and have associated exposure to these compounds with direct effects on physiological mechanisms associated with gonadal development and function.[32]

AQ:1 During pregnancy women are exposed to mixtures of EDCs, in exposures correlated to common sources as, serum levels of individual PCB congeners are correlated with one another as well as with some organochlorine (OC) pesticides.[33] Relevantly, the transplacental transfer of EDCs including

persistent organic pollutants (POPs; which include PCBs, OCs and flame retardants) and arsenic,[34] has been reported as well as the presence of relatively high concentrations of bisphenol A (BPA) in human placental samples and in fetal serum. Studies have demonstrated that the placenta does not work as a barrier to EDCs and, consequently, human fetuses are chronically exposed to these compounds.[35] Moreover, during early embryonic development, potential EDCs' targets include cell cleavage and differentiation, cell lineage determination, methylation, implantation, maintenance of pregnancy and organogenesis,[32] thus the determination of effective and valuable biomarkers of effect is of crucial significance.

18.4.1.1 Biomarkers of Effect for Pregnant Women

- Epigenetic markers in placenta

  In utero exposure to xenoestrogens, a sub-category of the EDCs group that are specifically associated with estrogen-like effects, can alter the placenta epigenome in a process in which male descendants appear to be more vulnerable to the effect of exposure to xenoestrogens during prenatal development, associated with shifts in DNA methylation of sensitive genomic repetitive sequences.[36] Additionally, some suggestive genes previously associated with birth weight, Type 2 diabetes, obesity or steroid hormones signaling were differentially methylated in males in relation to prenatal exposure to those compounds.[37]
- DNA damage biomarkers

  Prenatal exposures to nonylphenol (NP) resulted in the significantly increase of biomarkers of DNA damage namely, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-NO_2Gua) levels and increased maternal urinary 8-OHdG was correlated with significantly shorter gestational duration (~~adjusted~~ $\frac{1}{4}$ 4.72 days; 95% confidence interval (CI): ~~8.08–1.36~~ days). The authors suggested that prenatal 8-OHdG levels might be a novel biomarker for monitoring fetal health associated with NP exposure[38] (Table 18.1).
- Maternal hormone and metabolite levels

Phenols and parabens were associated with altered thyroid function during pregnancy, and, interestingly, the timing of exposure was positively associated with hormone concentrations which may result downstream in significant maternal and fetal health outcomes[39] (Table 18.1).

The relationship between urine phthalate metabolites and concurrent metabolomic markers in plasma and urine indicates a potential involvement of different pathways, such as lipid, steroid and nucleic acid metabolism and enhanced inflammatory response, in exposure outcome. Regarding urine phthalate metabolites, the authors reported nine positive significant associations with nicotinamide mononucleotide, cysteine T2, cystine and L-aspartic acid and also negative associations of maternal pre-pregnancy body mass index (BMI) with more than 20 phthalate metabolomic markers

Table 18.1 Biomarkers of effect for EDCs exposure during embryonic development.

EDCs	Studied individuals	Effect biomarkers	Samples	Reference
Nonylphenol (NP)	146 pregnant women	8-hydroxy-2′-deoxyguanosine	Urine	[38]
Phenols and Parabens	317 pregnant women	Thyroid hormone	Urine and blood	[39]
Phthalate metabolites	115 pregnant women	Metabolomic markers	Urine and blood	[40]
NP and BPA, PAEs and OP pesticide metabolites	162 pregnant women	Infant birth weight, length and head and chest circumference	Urine	[41]
PFASs and organochlorines (OCs)	424 mother–child pairs	Fetal growth	Urine	[42]
BPA	982 pregnant women	Anogenital distance	Urine	[44]
Phthalate metabolites	85 pregnant women	Anogenital distance	Urine	[45]
Phthalate metabolites	739 pregnant women	Anogenital distance	Urine	[46]

related to lipid and amino-acid metabolism and inflammation pathways in blood[40] (Table 18.1).

18.4.1.2 *Biomarkers of Effect in the Offspring After* in utero *Exposure*

- Fetal growth and development

  It has been demonstrated that the concurrent exposure throughout pregnancy to two phenolic EDCs (NP and BPA), nine phthalates (PAEs) and six organophosphorus (OP) pesticide metabolites affects infant birth weight, length and head and chest circumference. In a study performed in Taiwan urinary concentrations of the described EDCs were measured in urine samples from 162 women at approximately 11 (first trimester) and 26 weeks (second trimester) gestation and at delivery (third trimester). The authors reported significant relationships between mono-methyl phthalate (MMP, a phthalate metabolite) exposure and short birth length, second trimester total PAEs (ΣPAEs) and short birth length, second trimester ΣPAEs exposure and reduced head and chest circumference, second trimester diethylphosphate (DEP, an OP metabolite) exposure and decreased birth weight and length and second trimester ΣDEPs and short birth length. Also, women with short birth-length infants had significantly higher urinary NP levels in the second trimester, while infants with significantly reduced head circumference were positively correlated with urinary BPA above the 75th

percentile or ΣPAEs levels above the 50th percentile in the third trimester (Table 18.1).[41] Additionally, in a case–cohort study of 424 mother–child pairs in Norway (Trondheim and Bergen) and Sweden (Uppsala), authors reported that prenatal exposure to perfluorooctanoate (PFOA), PCB 153 and hexachlorobenzene (HCB) were correlated with higher odds for small-for-gestational-age (SGA) birth, particularly among Swedish male offspring[42] (Table 18.1). In addition to the described effects on neonate's physiognomy, morphology and phenotype, these results are concerning as infants with decreased birth weights are more susceptible to kidney dysfunction in adulthood and in addition to the gestation length are associated with indices of combined kidney biomarkers in infants.[43]

Moreover, infants' anogenital distances (AGDs) [AGDap (anus–penis) and AGDas (anus–scrotum) for boys, AGDac (anus–clitoris) and AGDaf (anus–fourchette) for girls] a biomarker that allows the assessment of fetal androgen action, were associated with maternal exposure to BPA and phthalate metabolites (Table 18.1). Male offspring from women with higher BPA levels had shorter AGDap and AGDas particularly by 12 months (2.87 and 4.12 mm shorter, respectively), while for female infants, associations were not found, indicating a higher male vulnerability.[44] Maternal urinary concentrations of phthalate metabolites [mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP) and mono-isobutyl phthalate (MiBP)] were also significantly associated with decreased AGI.[45] Furthermore, maternal urine levels of MEHP, mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl (MEHHP) phthalate at the first trimester were also inversely associated with male offspring AGD.[46] Considering that in males, short AGD is highly correlated with genital malformations and reproductive disorders in adulthood these results are particularly concerning and indicate that AGD should receive attention as a biomarker of choice for EDCs, effects on male reproductive health.[47]

18.4.2 Early Post-natal Life

It is currently acknowledged that early life experiences can result in health outcomes later in life according to the Developmental Origins of Health and Disease (DOHaD) hypothesis.[48] In addition to the early body burden of contaminant EDCs in newborns, derived from *in utero* exposure, cumulative data emphasize the crucial role of early post-natal life environment.[49]

Neonatal EDCs exposure is particularly associated with breast milk, as mixtures of lipophilic POPs (PCBs, brominated flame retardants, dioxins) can be transferred from the maternal body burden to the newborn through breastfeeding; in fact, breast-fed infants are among the population groups with high intake of PBDE.[50] The contamination of items utilized for nursing and artificial milk, can also determine the exposure of newborns and infants. A worldwide biomonitoring study based on data from urinary

BPA concentrations indicates that infants are the most exposed as estimated human exposure was 0.27 $\mu g\,kg^{-1}$ body weight per day for the general population, 0.78 $\mu g\,kg^{-1}$ body weight per day for children and 0.45–1.61 $\mu g\,kg^{-1}$ body weight per day for infants.[51] Although the EFSA has recurrently determined that the exposure levels in Europe are of no concern for human health,[52] in 2011 the European Legislation banned the use of BPA in the manufacture of baby nursing products.[53] Moreover, given the fact that internal defenses of neonates and infants against contaminants are limited by the immaturity of both hepatic detoxification and the blood–brain barrier, EDCs exposure can endorse severe health outcomes.[54] However, currently there are still no validated biomarkers of effect to assess risk exposure in infants and children and to evaluate the effects of those exposures in puberty and adult life and particularly fertility-associated disfunctions.

18.4.3 Puberty

Puberty is a stage of dramatic change through which an immature body matures into an adult body capable of sexual reproduction, initiated and controlled by hormonal signals from the brain to the gonads. Considering the crucial role of hormones in pubertal development, EDCs exposure may result in significant health outcomes and affect puberty processes such as puberty timing (Table 18.2). Interestingly, several epidemiological studies regarding EDCs' effects during embryonic development and early life, discussed above, indicate that males appear to be more vulnerable to EDCs exposure.[51,55] Additionally, the severe increase in male reproductive problems, which cannot be explained by genetic changes only, has occurred contemporaneously with cumulative exposures to numerous environmental factors associated with the modern lifestyle, including EDC mixtures which have been correlated with male reproductive disorders and diseases.[55] As an example, in a published review by Obaid Faroon and Patricia Ruiz that analyzed animal and human data cited in the US National Library of Medicine from 2000 to 2010, the authors emphasized that in animal studies PCB exposure has been associated with increased AGD and prostate size and with diminished epididymal weight, epididymal sperm count and motile epididymal sperm count.[56]

- Pubertal timing

  Timing of pubertal onset has been correlated with cardiometabolic markers in females (early pubertal timing associations with increased blood pressure)[57] and is suggested to be a marker of male reproductive health.[58] Results from several studies indicate that EDCs exposure effects on pubertal timing are sexually dimorphic, compound-specific and differ according to the window of exposure. While in girls divergent data have been reported, in boys pre-pubertal exposures to non-dioxin-like PCBs accelerate puberty while exposures to insecticides, dioxin-like compounds, OC pesticides and lead delay puberty[59] (Table 18.2).

Table 18.2 Biomarkers of effect for EDCs exposure at puberty.

EDCs	Studied individuals	Effect biomarkers	Samples	Reference
BPA	172 boys (9–11 years of age)	Serum hormones	Urine	63
PCBs insecticides, dioxin-like compounds, organochlorine pesticides, lead	159 boys and 179 girls (9–13 years of age)	Pubertal timing	Blood	59
MBP, *t*-OP, *n*-NP, daidzein, equol and genistein	Precocious puberty patients	Pubertal timing	Blood	60
Phthalate metabolites and BPA	338 children (9–13 years of age)	Pubertal timing	Urine	61
Dioxins, PCBs, chlorinated pesticides and lead	516 boys (8–9 years of age)	Pubertal timing	Blood	62

Additionally serum levels of the phthalate metabolite MBP, *t*-octylphenol (*t*-OP), *n*-NP, of the phytoestrogens daidzein, equol and, particularly, genistein were higher in patients with precocious puberty than in the controls, indicating a potential role in this condition[60] (Table 18.2).

In particular, phthalate metabolites, monocarboxynonyl phthalate, monocarboxyoctyl phthalate, mono (3-carboxypropyl) phthalate and BPA correlate with later pubarche and menarche, particularly among normal-weight girls, monobenzyl phthalate (MBzP) was associated with later thelarche and di(2-ethylhexyl) phthalate (PDEHP) was correlated with later thelarche and menarche in girls. On the other hand, BPA and all phthalate biomarkers of exposure were associated with earlier gonadarche and pubarche in boys[61] (Table 18.2). Additionally, dioxin-like compounds (cumulative exposure measured by oxic equivalents (TEQs)), OC pesticides and lead may delay, while non-dioxin-like-PCBs may advance, male puberty timing. Available data also indicate that persistent OCs and lead can negatively affect male growth during puberty[62] (Table 18.2).

- Biomarkers of male disfunction

  The association of serum hormone levels, namely testosterone (TT), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and cortisol, with urinary BPA concentrations was assessed in boys at 9–11 years of age. Increased urinary BPA levels were correlated with higher serum TT levels and decreased serum cortisol levels and also with higher TT:LH and TT:cortisol ratios.[63] On the other hand, besides exposure to man-made compounds with endocrine-disrupting effects, natural EDCs are also present in human food, such as soy phytoestrogens (*e.g.* genistein), and urinary phytoestrogen levels have been

positively associated with decreased percentages of normal sperm and augmented abnormalities in semen morphology.[64] Evidence of altered serum hormones at puberty, relevant for male fertility, and data from adult individuals regarding decreased sperm quality associated with urinary concentrations of EDCs clearly demonstrate a cumulative bioburden of EDCs that may contribute to male dysfunction. Concurrent with accumulating evidence from studies indicating that EDCs may contribute to male reproductive disorders and diseases, the burden of male reproductive health problems is significant not only at the individual and population levels but also in economic contexts. Prevention of EDCs exposures has the potential to reduce the incidence of several male reproductive disorders and diseases, their associated health care and other social costs.[55]

18.5 EDCs Occupational Exposure and Effects

Of the 538 papers found and evaluated in total for preparation of this chapter, 526 were excluded from the present review due to exclusion criteria and 11 were retained and included in a more detailed analysis concerning the occupational setting studied, EDCs considered, biomarkers of effect used and main conclusions (Table 18.3).

Different types of biomarkers of effect were used in the studies considered. In 10 studies (90.9%) it was possible to observe, at least limited, evidence of associations between exposure to EDCs and health effects.

18.6 Conclusion

Nowadays, modern living is filled with products and utensils consisting of new materials, which make our everyday lives easier and safer. Despite that, obvious benefits must not make us blind to the fact of these new chemicals, and their mixtures, can be harmful to endocrine systems of consumers and workers.

Current debates on human health risks related to EDCs still revolve around whether enough evidence is available that human exposure levels pose a risk to health. Until now, it has been important that animal testing includes relevant, sensitive endocrine-related endpoints and exposures occurring at time points relevant for developmental windows sensitive to endocrine action plus late manifestation of early life exposures. The reference values or tolerable daily intake values for individual chemicals with known endocrine disruptor abilities should be based on animal studies of enough sensitivity for detecting relevant endocrine-related endpoints and also study protocols should be flexible in order to evaluate potential mixture effects. In general, read across assessment within and between compound groups should be encouraged.

A goal in the use of biomarkers must be to identify adverse effects of chemical contaminants at the lowest levels of biological organization

Table 18.3 EDCs occupational exposure and biomarkers of effect.

Occupational setting	EDCs	Biomarkers of effect used	Main conclusions	Reference
348 job titles considered according to the Categories of Occupation from 1980.	Pesticides, polychlorinated organic compounds, phthalates, alkyl-phenolic compounds, biphenolic compounds, heavy metals and other substances.	Preterm birth and low birth weight.	No indications of reduced birth weight or increased risk of preterm birth were found among women potentially exposed to EDC.	65
Occupations coded according to the International Standard Classification of Occupations of 1988 (ISCO88; http://www.ilo.org/public/english/bureau/stat/isco/isco88/)	10 EDC groups as assessed through a job exposure matrix.	Birth weight, term low birth weight (LBW), length of gestation and preterm delivery.	Results indicate that employment during pregnancy in occupations classified as possibly or probably exposed to EDCs was associated with an increased risk of term low birthweight.	66
Occupational exposure to groups of EDCs was evaluated by a specific job exposure matrix (IEM) developed by Van Tongeren *et al.* (2002) for the purpose of classifying jobs in relation to exposures to EDCs.	~~S DDT~~, hexachlorobenzene and several polychlorinated biphenyl congeners.	Hypospadias in the offspring.	Evidence of an association between maternal exposure to EDCs, in particular with hexachlorobenzene and the development of hypospadias in the offspring.	67
Maternal and paternal professions with potential for EDC exposure were taken from the validated job–exposure matrix published by Van Tongeren *et al.,* (2002).	Paints, solvents, adhesives, detergents, pesticides, cosmetics and other industrial chemicals including metals, polycyclic aromatic hydrocarbons and herbicides.	Hypospadias.	Strongly indicates that EDCs are a risk factor for hypospadias through occupational and environmental exposure during fetal life.	68

Table 18.3 (*Continued*)

Occupational setting	EDCs	Biomarkers of effect used	Main conclusions	Reference
Cleaners, laboratory technicians, hairdressers and agricultural workers	Seven groups of EDCs: pesticides, organochlorine compounds, phthalate esters, alkyl phenols, bisphenols, heavy metals (cadmium, lead, mercury) and other compounds (hormone disrupting chemicals).	Hypospadias and cryptorchidism.	Limited evidence that occupational exposure to possible EDCs during pregnancy increases the risk of hypospadias.	69
Used a job exposure matrix developed by van Tongeren *et al.* 2002.	Categories of EDCs included pesticides, polychlorinated organic compounds, phthalates, alkyl phenolic compounds, bi-phenolic compounds, heavy metals and hormones such as phytoestrogens and synthetic hormones (oral contraceptives).	Hypospadias.	Evidence of an association between exposure to EDCs with estrogenic or anti-androgenic properties and increased risk of hypospadias.	70
Welders and smelters	Manganese.	Serum hormones level.	Disrupts the endocrine system and its balance	71
Agriculture, industry, cleaners and hairdressers	Pesticides, chlorinated insecticides, dicarboximide, fungicides, bisphenol A, polychlorinated biphenyls, dioxins, phthalates and parabens.	Hypospadias and cryptorchidism.	Besides some sociodemographic aspect also some parental occupational exposure to EDC, may increase the risk and predict the developments of these malformations.	72

Manufacturers of epoxy resins	BPA	DNA hydroxymethylation in human semen samples.	BPA exposure was associated with alterations of sperm long interspersed element 1 (LINE-1) hydroxymethylation.	73
Welders	Heavy metals and welding fumes.	Testicular germ cell tumor (TGCT) in offspring.	Evidence of associations between parental exposures to heavy metals or welding fumes and TGCT in offspring.	74
BPA manufacturer and three epoxy resin manufacturers	BPA	Male sexual function.	Evidence that exposure to BPA in the workplace could have an adverse effect on male sexual function.	75

(molecular, sub-cellular), so to predict (and possibly prevent) toxicological problems at a higher biological organization stage.

Biomarkers that have been validated for their predictive value may be used for the timely identification of increased cancer risk and can be used in the prevention or control of disease. The assumption underpinning the use of a biomarker as a surrogate for disease is that the observed relationship between exposure and the marker will translate into a similar relationship between exposure and disease.

In general, biomarkers of effect are very sensitive and the advantages of using biomarkers as tools for exposure assessment are well established. Biomarkers are particularly useful when their toxicological significance is sufficiently understood, including the following: toxicokinetic fate of the chemical or its metabolites (for exposure biomarkers), or the mechanism of disease or adverse effect (for effect biomarkers), or the modulating factors linking the chemical to the disease or adverse effect (for susceptibility biomarkers).

The global and continuous massive production and consumption of items containing EDCs results in a rising number of individuals exposed to mixtures of these compounds each day, including pregnant women and young children. Despite this, the risk to human health endorsed by the 'body burden' of contaminants is still insufficiently, or even not managed, by regulation in different world areas, or even routinely monitored. Nevertheless, in recent year's epidemiological studies have demonstrated EDCs' exposure outcomes through several biomarkers of effect, which support the urgency to perform valuable and accurate risk assessment in order to protect exposed individuals and their descendants, from the hazardous effects of aggregate EDCs mixtures. Indeed, the results of analyses performed in occupational studies indicated that exposure to EDCs mixtures, the most common exposure scenario for a worker due to aggregate and cumulative exposures, is cause of several health effects on the workers exposed and their offspring. This finding supports the need for developing more human biomonitoring studies, specifically on occupations recognized as linked to exposure to multiple EDCs. Additionally, health outcomes in offspring should also be followed during childhood.

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