Developmental toxicity of endocrine-disrupting chemicals: Challenges and future directions

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Abstract

Maternal exposure to a mixture of various endocrine disruptors (EDCs) may have a substantial impact on postnatal health of her offspring(s) and increase the risk for health disorders and diseases in adulthood. Research efforts to better understand the health risk associated with endocrine disruptor exposures in early life have increased in recent decades. This paper provides a short overview of the current challenges that researchers continue to face in selecting appropriate epidemiologic methods and study designs to identify endocrine disruptors and evaluate their adverse health effects during this critical developmental window. Major challenges involve the selection of a representative biomarker that reflects the foetal internal dose of the biologically active chemical or its metabolite(s) that may be associated with adverse health effects with regard to variable level and duration of exposure and the latency between exposure and disorder/disease manifestation. Future studies should pay more attention to identifying factors that contribute to interindividual variability in susceptibility to various EDCs and other toxicants.

Keywords: endocrine-disrupting chemicals, developmental origins of health and disease, epidemiologic research, biomonitoring, biomarkers

Introduction

The optimal functioning of the endocrine system is crucial for human health from the moment of our conception until the day we die. Produced in the endocrine glands, hormones operate in the body at extremely low levels, controlling crucial processes in the body. All endocrine organs depend on a delicate endogenous hormonal balance. Their concentrations can vary based on the age and physiological status of the individual, as well as circadian rhythms. Distributed through the body, hormones act as "chemical messengers" stimulating growth and development, regulating emotions and controlling body temperature, blood glucose levels, blood pressure etc. An imbalance of the hormonal system or their failure can cause adverse effects, including developmental, reproductive, neurological, and immune disorders in wildlife or humans (1-3).

Hormones have a particularly important role in the development and overall health of a child, and the developing embryo and/or foetus during pregnancy are highly vulnerable to any endogenous or exogenous hormone disturbances. Maternal stress, nutrition, and exposure to harmful substances during this critical period of life, when most of organs and tissues are formed and developed, can impair maternal and foetal health and cause adverse pregnancy outcomes (4-5). These effects may be evident years or decades later. Moreover, various pollutants and lifestyle factors have an important role in programming the susceptibility of offspring to several non-communicable diseases in their adult life (6-9).

It has been found that some chemicals could mimic, block, or interfere with the production, transport and general functioning of hormones in the body, altering the ability of hormones to bind to their native receptors, acting either as receptor agonists or as antagonists. These chemicals are referred to as endocrine-disrupting chemicals (EDCs). Depending on the time and duration of exposure, chemical characteristics and dose, EDCs can lead to different outcomes through different mechanisms. Of particular concern are chemicals that affect hormone system homeostasis impairing implantation, placentation, vascular remodelling, immunomodulation, and labour. Through interferences with sex steroid hormones, these chemicals may affect embryonic and foetal development, sex differentiation, pubertal timing and development (10). Androgens produced by the foetal testes are essential for the development of the male phenotype (11). It has been suggested that prenatal exposure to compounds with oestrogen-like activity or other environmental factors may be responsible for the observed decrease in semen quality and increased incidence of genitourinary abnormalities such as cryptorchidism, hypospadias and testicular cancer in several countries over a 50-year period (3,12-14). Disruption of maternal thyroid hormone levels during pregnancy and especially during the first trimester, when the foetus relies entirely on transplacental transfer of maternal thyroid hormones and thus on a normal maternal thyroid function, may have deleterious effects on neurological and other thyroid-related developmental outcomes (15-16). Both "too much" and "too little" hormones can contribute to subtle functional changes during early life that may alter cell- and tissue-specific gene transcription and expression and could be transmitted transgenerationally (17-18).

In order to take appropriate protection measures, it is necessary to identify the chemicals that can cause adverse health effects, assess the range of human exposure to these chemicals and understand the underlying mechanisms. Although research efforts to better understand the human health risk associated with EDCs exposure in early life have intensified over the last decades, researchers are still facing different pitfalls and challenges regarding the epidemiologic design and methods used to identify and evaluate EDCs. Low-level exposure effects, non-monotonicity, latency between exposure and disease/disfunction and unpredictable multi-component mixture effects further complicate the study of EDCs. This article aims to provide a short overview of the current challenges of epidemiologic research of developmental toxicity of endocrine-disrupting chemicals.

Endocrine-disrupting chemicals (EDCs): definition and identification

Despite the continuous progress in understanding endocrine disruption, identification of EDCs and risk assessment of their adverse health outcomes may be very difficult. The scientific criteria for the determination of endocrine disruption properties of active substances on human health could be based on defining what an EDC is. There are several definitions of EDCs proposed by several agencies (1,19-21). As a result, different cut-offs in terms of "adverse effects" can be established for the evidence required to determine whether a compound is an EDC (1,22-23). According to the widely accepted definition proposed by the World Health Organization (WHO), EDC is "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (24-25). The WHO also defines "a potential endocrine disrupter (as) an exogenous substance or mixture that poses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations" (25). Accordingly, the "identification of a substance as an endocrine disruptor should be based on an adverse effect for which a) there is convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action; and b) disruption of the endocrine system is not a secondary consequence of other non-endocrine-mediated systemic toxicity" (26).

Regulatory agencies use various approaches to evaluate the available evidence for EDC identification. However, different methods, analyses of different end points and application of different cut-offs in these evaluations result in different conclusions for hazard identification (27-28). To improve EDC characterization and identification and reduce their misclassification, a group of experts recently created 10 "key characteristics" of EDCs (29). The proposed key characteristics provide standard and systematic approaches that can be used to better organize the relevant literature on mechanistic data, improve the prioritization of chemicals for regulatory action, identify biological pathways that require additional assays (to be developed if necessary), and build public health-protective cases against the worst compounds (29-30).

Heterogeneity of EDCs

The group of chemicals identified as endocrine disruptors is highly heterogeneous. A wide range of synthetic and natural chemicals commonly used for a number of industrial and household products, personal care products, electronics, pharmaceuticals and medical devices are suspected to be EDCs. Some of them, such as pesticides, herbicides and antimicrobial agents, are designed to be biologically active and to alter an endocrine pathway in target species, but they may have unintended effects on non-target species. According to the Endocrine Disruptor Exchange list (TEDX), over 1400 chemicals have been suspected to have endocrine-disrupting effects and thousands of new chemicals with no-known potential endocrine disruption enter the marketplace each year (31). Some of the most frequently studied chemicals recognized as endocrine disruptors are diethylstilbestrol (DES), dichlorodiphenyltrichloroethane (DDT), vinclozolin, organophosphate pesticides, phthalates, bisphenol A (BPA), alkylphenols, polychlorinated biphenyls (PCB), dioxins, polycyclic aromatic hydrocarbons, brominated flame retardants, and metals such as cadmium, mercury, arsenic, lead and nickel (31-32). It has been observed that many associations between these compounds and adverse health outcomes demonstrate sex-specific effects. Thousands of scientific papers that linked adverse health effects to EDC exposure have been published. Table I presents examples of epidemiologic findings for adverse effects of some EDCs during pregnancy.

Table I   Examples of epidemiologic findings for adverse effects of endocrine disruptor chemicals (EDC) exposure during pregnancy (*in utero*).

Tabela I   Primeri epidemioloških nalaza štetnih posledica izloženosti navedenim endokrinim ometačima tokom trudnoće (in utero).

EDC	Observed effects	Study details		Reference
		Study population	Results	
FEMALE EFFECTS				
DES	Increased risk of vaginal epithelial changes including adenosis	Four USA cohorts (DESAD project), 2940 DES exposed daughters before GW 18	The highest prevalence among those exposed to >2300 mg DES	33,34
DES	Increased risk of menstrual irregularity	The US National Cancer Institute DES Third Generation Study; women whose mothers were prenatally DES-exposed (N=381) and unexposed (N=280)	Comparing the DES-exposed to the unexposed, PR (95% CI): 1.32 (1.10, 1.60) at age 15 or later.	35
	Increased risk of amenorhea		PR (95% CI): 1.26 (1.06, 1.49)	
	Increased risk of preterm birth		RR (95% CI): 1.54 (1.35, 1.75)	
	Increased risk of ectopic pregnancy		6.00 (0.45, 78.3)	
DES	Endometriosis	One family from Canada, DES-exposed daughters, subsequent granddaughters, unexposed daughter, and her progeny. The mother was prescribed DES 30 mg/day for 3 months after the birth of each of her 11 children (4 boys + 7 girls).	Only DES-exposed daughters (N=6) and subsequent granddaughters have endometriosis.	36

EDC	Observed effects	Study details		Reference
		Study population	Results	
FEMALE EFFECTS				
DES	Increased risk of breast cancer	Four USA cohorts (DESAD project) with 4817 exposed and 2073 unexposed daughters; follow-up study (since 1978 and 1994)	The overall age-adjusted IRR (95% CI) 1.40 (0.89, 2.22)	37
DDT	Increased risk of breast cancer	A 54-year follow-up study of 9300 daughters in California, USA (Child Health and Development Studies pregnancy cohort) which mothers were pregnant between 1959 and 1967. N=118 breast cancer cases by age 52 y and 354 controls matched on birth year. Breast cancer cases were identified btw. 2010 and 2013.	Elevated maternal serum o,p'-DDT significantly predicted a nearly 4-fold increase in the daughter's risk of breast cancer: OR (for Q4) (95% CI): 3.7 (1.5, 9.0), p=0.004. Maternal serum o,p'-DDT concentration, median (IQR), ng/mL: cases 0.52 (0.32, 1.06) control 0.46 (0.27, 0.78).	38
Phthalate esters and metabolites	Premature breast development (thelarche) – premature sexual development	Puerto Rican girls, 41 with thelarche (aged from 6 months to 8 years) and 35 controls (aged from 6 months to 10 years). Samples taken from Jan 1994 to Apr 1998.	High levels of serum phthalate and its major metabolites (DBP, DEP, DEHP, MEH) detected in 28 (68%) girls with thelarche. DEHP levels were significantly higher in cases than in control.	39
Phthalate esters and metabolites	Associated with decreased anogenital index in newborn girls	Pregnant women who planned to undergo amniocentesis during 2005 and 2006 in Taiwan. N=33 girls at birth	Amniotic fluid MBP (median and range, ng/mL): 85.5 (39.3-192.0)	40
Cd	Associated with preterm low birth weight	The Healthy Baby Cohort (HBC) during 2012 and 2014 in China, 408 mother-infant pairs. Cases (N=102): preterm low birth weight (PLBW) mothers of a live singleton infant with a gestational age <37 weeks and weighing <2500 g. Controls (N=306): mothers of a singleton live infant with gestational age ≥37 weeks and weighting btw. ≥2500 g and <4000 g. Female infants=180. Maternal urine collected within 3 days before delivery (third trimester).	Maternal urine Cd (UCd), median (range), μg/g creatinine Cases: 0.60 (<LOD, 5.61) Control: 0.48 (<0.04, 18.09) P<0.05 A significant positive association for higher UCd and the risk of PLBW (adjusted OR=3.65 (95% CI 1.12, 11.88) for tertile T2 and OR=5.90 (95% CI 1.57, 22.23) for T3. Maternal UCd: T1: <0.35 μg/g creatinine T2: 0.35-0.70 μg/g creatinine T3: UCd ≥0.70 μg/g creatinine	41

EDC	Observed effects	Study details		Reference
		Study population	Results	
MALE EFFECTS				
DES	Increased incidence of epididymal cysts	Trial study (5 mg/day at GW 7, increased by 5 mg/day every second week up to maximum daily dose of 150 mg by 34 weeks), 25 years after: 308 DES-exposed and 307 placebo-exposed sons.	Epididymal cyst incidence 20.8% in exposed group *vs.* 4.9% in placebo	42
	Increased incidence of testicular hypoplasia		Testicular hypoplasia incidence 8.4% in exposed group *vs.* 1.9% in placebo group	
DES	Increased risk of hypospadias	A cohort with fertility problems (OMEGA) diagnosed in the Netherlands btw. 1980 and 1995. N=205 sons of mothers exposed to DES and N=8729 sons of non-exposed mothers	PR (95% CI) 21.3 (6.5, 70.1) Absolute risk of hypospadias for the sons of DES-exposed women is low.	43
DES	Increased risk of hypospadias	240 sons of exposed mothers and 17393 controls born in France btw. Jan 1993 and Dec 2002	PR (95% CI) 4.99 (1.2, 16.8)	44

EDC	Observed effects	Study details		Reference
		Study population	Results	
MALE EFFECTS			
DES	Increased risk of hypospadias	583 cases (sons of mothers probably or certainly exposed to DES) and 251 controls, born in the Netherlands btw. 1987 and 1997	OR (95% CI) 2.3 (0.7, 7.9)	45
DES	Increased risk of cryptorchidism	Three cohorts of men exposed to DES in utero (a study of the US National Cancer Institute); 1197 DES-exposed and 1038 unexposed men	RR (95% CI) 1.9 (1.1, 3.4)	46
	Increased risk of epididymal cyst		RR (95% CI) 2.5 (1.5, 4.3)	
	Increased risk of testicular inflammation /infection		RR (95% CI) 2.4 (1.5, 4.4)	
DDT, DDE	Hypothyroidism	Women presenting for delivery in Belgium btw. Aug 2013 and March 2016 and their newborns. Gestational age ranged from GW 34 to GW 42. N=113 mother-sons pairs. TSH measured in cord blood 3 days after birth.	A significant reduction of the TSH in boys having detectable cord blood 4,4'-DDE level compared to those with no detectable DDE level (β=-0.236, p=0.025).	47
HCB	Increased risk of hypospadias	The Southern Sweden Maternity Cohort (SSMC) contain serum samples collected in early pregnancy (GW 12-14) among women in Sweden. N=237 SSMC mothers of boys with hypospadias in year 1986-2002 and N=237 controls.	Highest exposure quartile was associated with higher risk of hypospadias OR (95% CI): 1.68 (0.92, 3.08) Maternal HCB levels, median (range), ng/mL: Cases 0.20 (0.01-1.26) Control 0.19 (0.01-3.25)	48
Dioxins	Decreased semen quality	39 sons born btw. Mar 1977 and Jan 1984 to Caucasian women who lived in the most dioxin-polluted areas near Seveso, Italy in 1976 and 58 controls. Maternal serum collected soon after plant explosion. Subgroups: breast-fed exposed group N=21, breast-fed control N=36; formula-fed exposed group N=18, formula-fed control, N=22.	Maternal serum TCDD levels, median (IQR), ng/L: At exposure (July 1976): 51.7 (26.6, 115.0) Extrapolated serum levels at conception of their sons: 26.0 (16.2, 58.9) Sons of exposed mothers had significantly lower sperm concentration (46.2 vs. 81.0 million/mL), total count (139.2 vs. 229.9), progressive motility (50.6 vs. 90.5%), and progressive motile count (50.6 vs. 90.5 million) than controls.	49
	Influence on reproductive hormones		Higher FSH and lower inhibin B in the breast-fed exposed group (N=21) than in the breast-fed control (N=36). Higher FSH and lower inhibin B in the breast-fed exposed group than in formula-fed exposed group (N=18).	
Phthalate esters and metabolites	Decreased anogenital index (AGI) in boys	A multi-centre pregnancy cohort Study for Future Families, Sept 1999 to Aug 2002; 85 mother-son pairs, infants age (12.6±6.9 months)	Inverse relationship between maternal prenatal urinary concentrations of phthalate metabolites (MEP, MBP, MBzP, MiBP) and AGI in their sons.	50,51
Phthalate esters and metabolites	Shortened anogenital distance (AGD) in boys	First trimester phthalate exposure; a prospective cohort study (The Infant Development and the Environment Study, TIDES), 2010-2012. N=365 male infants. AGD measured at birth.	Inverse relationship btw. maternal first trimester urinary phthalate metabolite concentrations (DEHP, MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCOP, MCNP, MCPP) and AGD in their male infants.	51,52

EDC	Observed effects	Study details		Reference
		Study population	Results	
MALE EFFECTS				
Phthalate esters and metabolites	Shorter AGD in boys	The Swedish Environmental Longitudinal, Mother and Child, Asthma and Allergy (SELMA) Study, a prospective birth cohort study. Pregnant women recruited in the GW 10 btw. Sept 2007 and March 2010 and their sons, N=196 boys born btw. 1. Sept 2009 and 20. Nov 2010, mean age=20.8 months	Maternal, a first morning urine measured for 10 phthalate metabolites. GM (95% CI) of DiNP metabolites (ng/mL): oh-MMeOP 6.81 (5.60, 8.28) oxo-MMeOP 3.05 (2.56, 3.63) cx-MMeHP 10.81 (9.16, 12.75) ΣDiNP 67.74 (57.00, 80.52)	53
PCB	Shorter AGI	The INfancia y Medio Ambiente Asturias (INMA) (Environment and Childhood) study, a population-based cohort of pregnant women in Spain, recruited btw. May 2004 and Jun 2007, GW 10-13. Maternal blood samples were collected. At deliveries btw. Oct 2004 and Feb 2008 cord-blood was collected. Follow-up at 4 years. N=116	Inverse association between AGI and maternal serum concentration of PCB-180 (β=-0.042, 95% CI -0.073, -0.011, T3) and cord serum PCB-153 (β=--0.047, 95% CI -0.085, -0.008, T3) and PCB-180 (β=--0.041, 95% CI -0.078, -0.005, T3). PCB concentrations, median (5th, 95th), ng/mL: Maternal serum PCB-180: 48.28 (13.09, 111.48) Cord serum PCB-153: 47.7 (18.64, 116.42) PCB-180: 27.79 (7.16, 68.85).	54
PFOA	Associated with impaired semen quality and reproductive hormone levels	A prospective study. Sons of a pregnancy cohort recruited in 1988 and 1989 in Denmark. Adult sons (19-21 y, N=169) undergo physical examination and gave a semen and blood sample in 2008-2009.	Multivariate analyses suggested negative trends for sperm concentrations and total sperm count and a trend of higher LH and FSH with higher prenatal exposure to PFOA. Maternal plasma PFOA, median (IQR), ng/mL 3.8 (2.8, 4.7)	55
Pb	Preterm birth	Mother-child pairs (N=949) in project VIVA, USA. Pregnant women at their first prenatal visit, < GW 22. Sample timing btw. 1999 and 2002. N=475 male children, N=35 preterm male children (< GW 37)	Maternal RBC Pb, mean ± SD, (μg/dL) (N=828): 1.22 ± 0.59 Mothers carrying male foetuses in the Q4 of RBC Pb (2.02 ± 0.60) have 5.5 times the odds of giving birth prematurely, compared to those in the lowest Q1 (0.65 ± 0.15): OR 5.51, 95% CI 1.21, 25.15.	56

EDC	Observed effects	Study details		Reference
		Study population	Results	
MALE AND FEMALE EFFECTS				
Pb	Preterm birth	The prospective Healthy Baby Cohort study, China. Longitudinal birth study performed during 2012 and 2014. N=7299 women, 283 with preterm birth (before GW 37), 2145 with early-term birth (GW 37 to 38) and 7016 with term birth (including women with early-term birth). Mean age of mothers: 28.51 ± 1.34, 54.7% aged 25 to 29 y. Urine samples were collected within three days before delivery.	A significant decrease in gestational age among all newborns with an increase in maternal urinary Pb (UPb) (β = -0.24, 95% CI -0.48, -0.00). Adjusted OR (95% CI): T2 1.43 (1.07, 1.89) T3 1.96 (1.31, 2.44). UPb, median (µg/g creatinine): All 2.97 Preterm-birth 3.44 Early-term birth 2.97 Term birth 2.95 Tertiles of UPb (µg/g creatinine): T1 (≤2.29), T2 (2.29-4.06) and T3 (>4.06). Average gestational age (GW): preterm 35.04 ± 1.34 early-term birth 37.81 ± 0.40.	57
Hg	Preterm birth	A cross-sectional study in pregnant women and their newborns in Indonesia conducted during January to June 2017. Preterm birth group: 26-36 gestation weeks, N=25 and term birth group N=25. Samples taken at delivery.	Significantly higher placental Hg (20.47 ng/g) of women with preterm delivery than women with term delivery (0.20 ng/g), p=0.019. No significant difference btw. the groups for Hg in maternal serum or cord blood samples.	58
Hg	Preterm birth, decreased birthweight	African-American pregnant women-newborns pairs from the Boston Birth Cohort, USA. Hg measured in paired maternal, umbilical cord, and postnatal blood samples (plasma and RBC).	Significantly higher Hg in maternal and cord plasma and RBCs in preterm or low birthweight births, compared with term or normal birthweight births. Hg, GM (95% CI), µg/L: Maternal RBCs 2.35 (1.82-3.03) Cord RBCs 3.58 (2.76-4.65) Maternal plasma 0.32 (0.24-0.42) Cord plasma 0.21 (0.16-0.29)	59
Cd	Associated with preterm low birth weight	The Healthy Baby Cohort (HBC) during 2012 and 2014 in China, 408 mother-infant pairs. Cases (N=102): preterm low birth weight (PLBW) mothers of a live singleton infant with a GW <37 and weighing <2500 g. Controls (N=306): mothers of a singleton live infant with gestational age ≥37 weeks and weighting btw. ≥2500 g and <4000 g. Male infants=228, female infants=180. Maternal urine collected within 3 days before delivery (third trimester).	Maternal urine Cd (UCd), median (range), µg/g creatinine Cases: 0.60 (<LOD, 5.61) Control: 0.48 (<0.04, 18.09) P<0.05 A significant dose-response relationship btw. UCd and risk of PLBW (adjusted OR=1.75 (95% CI 0.88, 3.47) for T2 and OR=2.51 (95% CI 1.24, 5.07) for T3.	60

EDC	Observed effects	Study details		Reference
		Study population	Results	
MALE AND FEMALE EFFECTS			
Cd	Sexually dimorfic effects	A randomised population-based food and micronutrient supplementation trial in pregnancy (MINIMat trial) involving pregnant women recruited from Oct 2002 through Oct 2003, Bangladesh. N=127 women-infant pairs. Maternal blood (and urine) collected at gestation weeks 8 and 14.	Low-level environmental Cd exposure during pregnancy is associated with sex-specific alterations in DNA methylation in foetal (cord) blood. Newborn boys showed generally global hypermethylation, girls showed markedly more hypomethylation. Maternal blood Cd associated with cord blood methylation in CpG sites in both male and female fetuses. Female fetuses: hypomethylation in around 40 genes associated with embryonic and organ development, bone morphology and mineralization → lower birthweight Male fetuses: hypermethylation in 105 genes related to cell death.	61
As	Sexually dimorfic effects	A population-based screening during 2002 and 2003, Bangladesh. As in drinking water ranged <1 to 3640 µg/L (WHO recommended limit is 10 µg/L). A randomized population-based food and micronutrient supplementation trial (MINIMat) in pregnancy. N=127 pregnant women delivered (N=65 girls and N=62 boys) from May 2003 to Jun 2004, non-smokers. Sum concentration of As metabolites (inorganic As, methylarsonic acid (MMA) and dimethylarsinic acid (DMA)) measured in spot urine samples collected btw. gestation weeks (GW) 5 and 14 (median GW 8) and btw. GW 26 and 36 (median GW 30). DNA methylation of gene-related CpG sites (500) determined in cord blood.	In GW 8, 277 (55%) of top 500 CpG loci methylation was decreased (hypomethylation) and 223 (45%) was increased (hypermethylation) with increasing As exposure in all infants. Effects much more evident in boys than in girls. Among boys, 372 (74%) of top 500 CpG sites showed hypomethylation with increasing As exposure, compared with 207 (41%) among girls (P<0.001). Maternal urinary As (UAs) median (95% CI), µg/L: GW 8: 66 (20-457) GW 30: 89 (18-562) Percent of total metabolite concentrations, mean ± SD Inorganic As: 13.8 ± 5.5 MMA: 10.06 ± 3.6 DMA: 76.12 ± 7.5	62

Abbreviations:

CI, confidence intervals; GM, geometric mean; LOQ, limit of quantification; OR, odds ratios; IRR, incidence rate ratios; RR, risk ratios; T1-T3, tertiles; Q1-Q4, quartiles; GW, gestation week; AGD, anogenital distance; AGI, anogenital index (anogenital distance/weight, mm/kg); DES, diethylstilbestrol; DDT, p,p′-dichlorodiphenyltrichloroethane; DDE, p,p′-dichlorodiphenyldichloroethylene; o,p'-DDT (an isomer of DDT); HCB, hexachlorobenzene; MEP, monoethyl phthalate; MBP, mono-*n*-butyl phthalate; MBzP, monobenzyl phthalate; MiBP, monoisobutyl phthalate; PFOA, perfluorooctanoic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; RBC, red blood cells, erythrocytes; TSH, thyroid stimulating hormone.

Skraćenice:

CI, intervali pouzdanosti; GM, geometrijska sredina; LOQ, granica kvantifikacije; OR, odnosi verovatnoća; IRR, odnosi stope javljanja; RR, odnosi rizika; T1-T3, tertili; Q1-Q4, kvartili; GW, gestacijska nedelja; AGD, anogenitalna udaljenost; AGI, anogenitalni indeks (anogenitalna udaljenost/težina, mm/kg); DES, dietilstilbestrol; DDT, p,p′-dihlordifeniltrihloretan; DDE, p,p′-dihlordifenildihloretilen; o,p'-DDT (izomer DDT); HCB, heksahlorbenzen; MEP, monoetil ftalat; MBP, mono-*n*-butil ftalat; MBzP, monobenzil ftalat; MiBP, monoizobutil ftalat; PFOA, perfluorooktanska kiselina; TCDD, 2,3,7,8-tetrahlordibenzo-*p*-dioksin; RBC, crvena krvna zrnca, eritrociti; TSH, tireostimulišući hormon.

Human exposure to EDCs

Humans are exposed to a mixture of various endocrine disruptors daily through air, dust, food and water, cosmetics, and food contact materials. Individuals working, for example, with pesticides, plastics and some industrial chemicals, can be exposed to substantially higher levels of EDCs at work. However, the general population are usually exposed to low levels of multiple EDCs constantly and inevitably by ingestion, inhalation, and dermal contact (25).

An increasing body of evidence supports associations between exposure to realistic environmental or dietary levels of these chemicals and endocrine-disrupting effects related to adverse health effects (2-3,21,63-64). These findings have challenged traditional concepts in toxicology, whose general goal is to establish a threshold dose below which a substance can be considered safe (the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL)). In addition to the adverse effects obtained at low-level exposure (2,65), several studies have reported non-monotonic dose-response curves (2,66-68) for a number of chemicals, including pesticides, PCBs, dioxins, and phthalates, mainly with regard to their endocrine activity (68-69). Non-monotonic dose-response (i.e. U-shaped or inverted U-shaped dose response curves) is defined "as a curve that changes slope from one direction to another at some point along the range of doses examined and therefore occur in any part of the dose response curve, not only at low doses" (2,66-68). Many natural hormones in the human body, such as steroid hormones, act at extremely low serum levels, typically within the picomolar to nanomolar range. Moreover, natural hormones also show complex dose-response, non-monotonic dose response curves (2,21,64). Therefore, it is logical to expect that EDCs also act at such low concentrations, disrupting the function of the endocrine system. Low-dose effects could be observed at the lower end of a monotonic or linear part of dose-response curve, but the likelihood of effect detection would be affected by the number and range of doses examined (70-71). Non-monotonic dose-response is a common and expected feature of chemical interferences with hormonal systems, and may be explained by different modes of action (MoA) operating at different dose-levels. The occurrence of response in different parts of the dose-response curve would have different impacts on risk assessment. The effects observed at high doses are often different from the effects observed at low doses of chemicals and it is very difficult to predict them from the non-monotonic dose-response relationship. Some scientists disagree with the phenomenon of low-dose response, U-shaped, or inverted U-shaped dose–response curves and emphasize the necessity for its verification (72).

Many EDCs are fat-soluble and adipose tissue is a particularly important reservoir for them. Some EDCs, such as PCBs, dioxin, and organochlorine pesticides, have long half-lives, while others, such as BPA and phthalates, are excreted from the body after only a few hours (16). EDCs may be metabolised to compounds that are more toxic than the parent chemicals (20,73).

These chemicals can interact and produce antagonistic, additive or synergistic effects on health beyond the impact from a single chemical acting in isolation. Chemical mixtures act through different mechanisms and can cause different biological changes and health effects compared to individual ingredients. Multiple exposures can result in cumulative effects for compounds acting via similar pathways and for compounds acting on similar health outcomes via different pathways. Synergistic effects can also be observed. Traditional risk assessment models applied to the exposure assessment of a single chemical may underestimate the realistic toxicity risks of a chemical mixture (74-75).

Harmful effects can be visible immediately or later in life, sometimes as a result of some other "hit". Confounding factors such as age, gender, general health and lifestyle that may influence individual susceptibility to health impairment add complexity to such an evaluation. Therefore, assessing risk from exposure to a huge number of multiple chemicals by multiple routes represents a substantial scientific challenge (74). A better understanding of the nature and magnitude of exposures to EDCs, their interactions at the target sites and the effects of combined and cumulative effects on health would provide valuable data relevant to risk assessments. However, an assessment of the risk from exposure to multiple EDCs that take into account real-life levels and multiple exposure routes is still a substantial problem in health evaluations.

EDCs act through multiple mechanisms, causing various biological changes and health effects. These mechanisms depend on the timing of exposure and the species exposed.

Pregnancy as a vulnerable exposure window

Reproductive age is a critical period in the life of a woman and her exposure to harmful stressors and development of pathological conditions during this period of life may impact the outcomes of future pregnancies, as well as the health of her offspring(s). Adverse effects on a developing organism may result from the exposure of either parent before conception, during prenatal development, or postnatally to the time of sexual maturation. It has been established that the life *in utero* has an important impact on embryonic and foetal development. Disorders during the early stages of a child's development can cause irreversible effects and represent a strong predictor for future health. The concept "developmental plasticity" proposes that an organism undergoing prenatal development or early life is able to change its phenotype to adapt to the environment. Such "foetal programming", as a developmental adaptation to the adverse intrauterine environment that produces permanent structural, physiological and metabolic changes, can increase the risk of developing a disease later in life. This concept is referred to as the "developmental origins of health and disease (DOHaD)" (6-8).

An important role in this programming is attributed to the placenta (76). This complex organ undergoes a number of changes during pregnancy, first to form the maternal-foetal interface (77-78), and later, as an endocrine organ, to produce and secrete hormones and other mediators essential for a normal pregnancy outcome (77,79). It is a

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dynamic organ whose molecular structure and function change during pregnancy (80). Via the umbilical cord, it provides the passage of nutrients and oxygen to the foetus and removes waste products back from the foetus to the mother. However, together with nutrients, many harmful substances such as lead, mercury, PCBs, can also cross the placenta and enter the foetal blood, impairing foetal health and development. Substances that do not cross the placenta may still harm the foetus through interaction with nutrient transporters or by interference with the hormonal system. For example, a smoking pregnant women exposes her developing embryo/foetus to a variety of chemicals from tobacco smoke (81), which contains a mixture of chemicals, including EDCs, with cadmium levels being among the highest. Cadmium disturbs the homeostasis of essential elements, such as zinc and copper and interferes with their placental transfer, alters placental steroidogenesis, and may affect the epigenetic mechanisms responsible for foetal programming during intrauterine life (82-86).

The placenta is able to adapt to the intrauterine environment in order to, among other things, provide adequate nutritional support for a developing foetus (78). Such an adaptation process includes epigenetic mechanisms. Induction of epigenetic changes is recognised as one of the mechanisms by which gestational exposure to EDCs increases the predisposition to various adverse health outcomes that manifest years or decades later. Epigenetic changes include changes in gene expression without modifications of gene code or DNA sequence itself, DNA methylation, histone modification, and non-coding RNA. Moreover, "reprogramming" of the genome in the germ line may potentially result in trans- and intergenerational effects since epigenetic changes may transmit to subsequent generations (18,87).

The epidemiologic challenges and perspectives

The maternal womb is the first environment for the developing offspring *in utero*, and the mother's diet, physiological status and exposure to various stressors have a substantial impact on her offspring. Our understanding of the relationship between environmental exposure to EDCs and human health outcomes in critical window(s) of development is highly challenged. Complex reproductive and developmental cycles, variable persistence of EDCs in the environment and in the body, non-monotonic dose-response, multiple exposures to a huge number of various chemical and lifestyle stressors, dose additivity and synergism between and among hormones and chemicals, as well as latency between the time of exposure and time of manifestation of adverse outcomes, complicate our understanding of the risk that EDCs pose (3,88).

Epidemiologic research allows for the study of realistic routes, levels, and duration of exposure relevant to realistic scenarios in humans, but epidemiologic studies are scarce and their results are often inconsistent. Methodological limitations, inadequate study designs and small sample sizes, variations in exposure levels, difficulties in the selection of representative biological matrices for exposure measurement from a specific population at an appropriate time, limited knowledge and control of potential confounders have all contributed to major sources of uncertainty in the epidemiologic evidence (73,88-

90). High analytical and collection costs, and potential participant selection bias additionally challenge epidemiologic research and biomonitoring of pregnant women and their offspring(s).

Most epidemiologic studies are cross-sectional, with the researcher measuring the selected outcome and the exposures in the study participants at a single point in time. As many of EDCs are persistent, a single measure of their concentration in a body fluid may be a suitable surrogate for long-term exposure. However, if a selected chemical has a short biological half-life and rapid clearance rate, it is very difficult to select the appropriate time for their measurement. A better understanding of the toxicokinetics (absorption, distribution, metabolism, and excretion) of various EDCs and their mixture, especially during pregnancy, will aid the researcher in selecting appropriate biomarkers and biospecimens within a proper timeframe (90). The selection of representative biospecimen, appropriate procedures for their collecting, processing and storing is an integral part of the validity of the biomarker. Concomitant analysis of maternal blood and urine, placenta and cord blood, together with data on the exposure sources, is the best way to assess exposure to EDCs and their disposition across the maternal-placental-foetal unit (91). Recent advances in new "omics" technologies have provided a comprehensive insight into the physiological and pathological events in the body and enabled a better understanding of the changes that occur. The application of metabolomics to epidemiologic studies of EDCs has the potential to better characterise exposures and detect early biomarkers of exposure and effects. Multidisciplinary and collaborative efforts are needed to create better screening tools to identify EDCs and validate their health risk.

Biomonitoring data reflects exposure from all sources and all routes and is indicative of real-life chemical exposures. It allows us to measure either the substances themselves or their metabolites or markers of subsequent health effects in body fluids and tissues. These measurements reflect current, past and cumulative exposure to the chemical(s) of interest. The selection of an appropriate biomarker that reflects the dose of the biologically active chemical at the target site for a relevant time of interest is essential for evaluation of health risk (90,92). It could be particularly important in studies of foetal development to select those biomarker(s) that reflect(s) foetal exposure, health effects and susceptibility. The foetal internal dose, i.e. the amount of EDC or its metabolite in developing organs or foetal blood would be a key factor of the risk of adverse effects. However, as it is not possible to collect foetal biospecimens during pregnancy, maternal EDCs concentrations during pregnancy and cord blood concentration at delivery are often used to assess foetal exposure over pregnancy or at term, respectively. Although the mother and her offspring live in the same environment, due to higher sensitivity of the embryo/foetus to adverse effects of EDCs, results are sometimes difficult to interpret.

Pregnancy is a dynamic process involving systemic and local changes in the mother and her offspring and adverse effects of EDCs can be detected at any point during pregnancy and later. If the relevant time-specific exposures are not adequately tested or

assessed, important early or delayed adverse health outcomes may not be detected. Study designs that assess exposures and outcomes at various stages of embryonal/foetal development are preferable. Large, longitudinal prospective studies beginning before or during very early pregnancy, with serial measurements during pregnancy and in early childhood, would establish the temporality of the association between EDCs exposure and developmental health effect(s) more clearly.

Future studies should pay more attention to identifying factors that contribute to interindividual variability in susceptibility to various EDCs and other toxicants. Pregnant and lactating women are prone to essential element (calcium, zinc, iron) deficiencies and therefore may absorb and retain more toxic chemicals from the gastrointestinal tract. For example, lead and cadmium can interfere with the metabolism of calcium, zinc, and iron and reduce their concentrations and bioavailability for transport to the foetus, where it is necessary for growth and development (93). Variations in genes are involved in metal disposition. Genetic polymorphism of metallothionein, a cysteine-rich protein ligand with roles in the zinc and copper homeostasis, scavenging free radicals and detoxification, may influence individual sensitivity to toxic metals (94). Therefore, whenever possible, epigenetic markers and specific gene polymorphism(s) in candidate genes should be determined as modifiers of the relationships of environmental exposure to EDCs and the susceptibility of the host.

Conflict of interests

None to declare.

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Toksičnost endokrinih ometača tokom perioda razvoja:
Izazovi i budući pravci

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Kratak sadržaj

Majčina izloženost brojnim hemikalijama koje mogu delovati kao endokrini ometači može imati značajan uticaj na postnatalno zdravlje potomstva i povećati opasnost od zdravstvenih poremećaja i bolesti u odrasloj dobi. Poslednjih decenija se ulažu dodatni napori da se bolje shvati rizik koji izloženost endokrinim ometačima nosi za ljudsko zdravlje. Ovaj rad daje kratak pregled izazova s kojima se istraživači i dalje suočavaju pri izboru odgovarajućih epidemioloških metoda i dizajna istraživanja kako bi identifikovali endokrine ometače i procenili njihovo delovanje na zdravlje tokom osetljivog perioda rasta i razvoja. Među najveće izazove ubraja se izbor reprezentativnog biomarkera koji odražava fetalnu internu dozu biološki aktivne supstance ili njenog metabolita koja se može povezati sa štetnim delovanjem na zdravlje, s obzirom na varijabilnost nivoa i trajanja izloženosti, kao i na postojanje vremenskog razmaka do pojave štetne posledice. Potrebno je da buduća istraživanja posvete više pažnje pronalaženju činilaca koji doprinose interindividualnoj varijabilnosti u osetljivosti na delovanje endokrinih ometača i drugih zagađivača, kao i razvoju zdravstvenih poremećaja i bolesti u odrasloj dobi.

Ključne reči: endokrini ometači, razvojno poreklo zdravlja i bolesti, epidemiološka istraživanja, biomonitoring, biomarkeri