sciensano

healthy all life long

DETERMINATION OF ENDOCRINE DISRUPTING COMPOUNDS IN HUMAN PLACENTA BY UPLC-ESI-MS/MS

I. Van Overmeire[1], K. Vrijens[2] , T. Nawrot[2,3], C. Vleminckx[1], J. Van Loco[1], T. Reyns[1]

[1]Sciensano, Chemical and physical health risks,1050 Brussels
[2]Center for Environmental Sciences, Hasselt University
3 Department of Public Health, Environment &Health unit, Leuven University

.be

Outline

- Introduction
- Objectives
- Methods
- Results
- Conclusion

Introduction EDCs

- Chemicals that interfere with normal hormone action
- Mostly man –made chemicals (pesticides, flame retardants, plastic additives, cosmetics, personal care products)
- Suspected to be associated with :
  - ❑ altered reproductive function in males and females
  - ❑ increased incidence of breast cancer
  - ❑ abnormal growth patterns and neurodevelopmental delays in children
  - ❑ changes in immune function

Source: WHO

Exposure to EDC

Food, dust, water

Inhalation

Skin

Transfer from pregnant women to developing fetus or child (placenta and breast milk)

Vulnerable populations

effect of exposures to EDCs may not become evident until later in life.

Background

sciensano

.be

REVIEW

EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals

A. C. Gore, V. A. Chappell, S. E. Fenton, J. A. Flaws, A. Nadal, G. S. Prins, J. Toppari, and R. T. Zoeller

Pharmacology and Toxicology (A.C.G.), College of Pharmacy, The University of Texas at Austin, Austin, Texas 78734; Division of the National Toxicology Program (V.A.C., S.E.F.), National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709; Department of Comparative Biosciences (J.A.F.), University of Illinois at Urbana-Champaign, Urbana, Illinois 61802; Institute of Bioengineering and CIBERDEM (A.N.), Miguel Hernandez University of Elche, 03202 Elche, Alicante, Spain; Departments of Urology, Pathology, and Physiology & Biophysics (G.S.P.), College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612; Departments of Physiology and Pediatrics (J.T.), University of Turku and Turku University Hospital, 20520 Turku, Finland; and Biology Department (R.T.Z.), University of Massachusetts at Amherst, Amherst, Massachusetts 01003

The Endocrine Society's first Scientific Statement in 2009 provided a wake-up call to the scientific community about how environmental endocrine-disrupting chemicals (EDCs) affect health and disease. Five years later, a substantially larger body of literature has solidified our understanding of plausible mechanisms underlying EDC actions and how exposures in animals and humans—especially during development—may lay the foundations for disease later in life. At this point in history, we have much stronger knowledge about how EDCs alter gene-environment interactions via physiological, cellular, molecular, and epigenetic changes, thereby producing effects in exposed individuals as well as their descendants. Causal links between exposure and manifestation of disease are substantiated by experimental animal models and are consistent with correlative epidemiological data in humans. There are several caveats because differences in how experimental animal work is conducted can lead to difficulties in drawing broad conclusions, and we must continue to be cautious about inferring causality in humans. In this second Scientific Statement, we reviewed the literature on a subset of topics for which the translational evidence is strongest: 1) obesity and diabetes; 2) female reproduction; 3) male reproduction; 4) hormone-sensitive cancers in females; 5) prostate; 6) thyroid; and 7) neurodevelopment and neuroendocrine systems. Our inclusion criteria for studies were those conducted predominantly in the past 5 years deemed to be of high quality based on appropriate negative and positive control groups or populations, adequate sample size and experimental design, and mammalian animal studies with exposure levels in a range that was relevant to humans. We also focused on studies using the developmental origins of health and disease model. No report was excluded based on a positive or negative effect of the EDC exposure. The bulk of the results across the board strengthen the evidence for endocrine health-related actions of EDCs. Based on this much more complete understanding of the endocrine principles by which EDCs act, including nonmonotonic dose-responses, low-dose effects, and developmental vulnerability, these findings can be much better translated to human health. Armed with this information, researchers, physicians, and other healthcare providers can guide regulators and policymakers as they make responsible decisions. (*Endocrine Reviews* 36: E1–E150, 2015)

1. Obesity & diabetes
2. Female reproduction
3. Male reproduction
4. Hormone senitive cancer
5. Prostate cancer
6. Thyroid
7. Neurodev & neuroendocrine system

sciensano

.be

Environmental Research 169 (2019) 131–138

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Preterm birth in relation to the bisphenol A replacement, bisphenol S, and other phenols and parabens

Max T. Aung[a], Kelly K. Ferguson[a,b], David E. Cantonwine[c], Thomas F. McElrath[c], John D. Meeker[a,*]

[a] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, United States
[b] Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States
[c] Division of Maternal and Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States

ARTICLE INFO

Keywords:
Preterm birth and subtypes
Phenols
Parabens
Repeated measures

ABSTRACT

Introduction: Preterm birth continues to be a significant public heath concern and is a leading cause of perinatal and infant mortality. Environmental exposures to phenols and parabens are suspected to potentially contribute to the pathology of preterm birth, yet limited human studies have characterized the extent to which these toxicants are associated with birth outcomes.

Methods: We examined the associations between phenols, parabens, and preterm birth, within pregnant women who were recruited early in gestation into the LIFECODES cohort at Brigham and Women's Hospital in Boston, Massachusetts. Urine samples were collected at up to 4 time points in pregnancy and analyzed for phenols and parabens. We selected 130 cases of preterm birth (defined as delivery before 37 weeks gestation), and 350 random controls. We categorized preterm birth subtypes based on clinical presentation and identified 75 cases of spontaneous preterm birth (characterized by spontaneous preterm labor and/or preterm premature rupture of membranes), and 37 cases of placental preterm birth (characterized by preeclampsia and/or intrauterine growth restriction). We used multivariate logistic regression with visit specific and geometric averages of phenols and parabens to determine associations with preterm birth.

Results: We observed moderate variability in urinary phenol and paraben concentrations over pregnancy with intraclass correlation coefficients ranging between 0.45 and 0.68. Regression analyses indicated mostly null

Reproductive Toxicology 59 (2016) 89–95

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias

Mariana F. Fernández[a,b,*], Juan P. Arrebola[a,b], Inmaculada Jiménez-Díaz[a,c], José María Sáenz[a], José Manuel Molina-Molina[a,b], Oscar Ballesteros[c], Andreas Kortenkamp[d], Nicolás Olea[a,b]

[a] Instituto de Investigación Biosanitaria (ibs.GRANADA), University of Granada, San Cecilio University Hospital, 18071 Granada, Spain
[b] CIBER en Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[c] Research Group of Analytical Chemistry and Life Sciences, Department of Analytical Chemistry, University of Granada, Granada, Spain
[d] Institute of the Environment, Health and Societies, Brunel University, Kingston Lane, Uxbridge, UB8 3PH, UK

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 12 October 2015
Accepted 2 November 2015
Available online 19 November 2015

Keywords:
Endocrine disrupting chemicals
Cryptorchidism
Hypospadias
Placenta
Foetal exposure
BPA
Parabens

ABSTRACT

Embryo-foetal exposure to low doses of endocrine disrupting chemicals (EDCs) has been related to reproductive tract diseases in experimental animals but not convincingly in human populations. The aim of this case—control study was to explore the relationship between exposure to non-persistent EDCs during pregnancy and male genital development. Exposure to bisphenol-A (BPA), benzophenones (BPs) [BP-1, BP-2, BP-3, BP-6, BP-8 and 4-hydroxybenzophenone (4-OH-BP),] and parabens (PBs) [methyl-, ethyl-, propyl- and butyl-PB] was analyzed by means of ultra-high performance liquid chromatography-tandem mass spectrometry in placenta samples from a subsample of 28 cases and 51 healthy controls nested in a cohort of newborns recruited between 2000 and 2002. The multivariable regression analyses indicated a statistically significant association between exposure to BPA and propyl-PB and the risk of malformations [adjusted odd ratio (95% CIs) in the third tertile of exposure: 7.2 (1.5–35.5) and 6.4 (1.2–35.5) for BPA and propyl-PB, respectively].

Environmental Research 164 (2018) 109–113

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Maternal use of personal care products during pregnancy and risk of testicular germ cell tumors in sons

Armen A. Ghazarian[a,b], Britton Trabert[a], Kim Robien[c], Barry I. Graubard[a], Katherine A. McGlynn[a,*]

[a] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA
[b] Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA
[c] Department of Exercise and Nutrition Sciences, Milken Institute School of Public Health, George Washington University, Washington, DC, USA

ARTICLE INFO

Keywords:
Testicular cancer
TGCT
EDCs
Endocrine-disrupting chemicals
STEED
Personal care products

ABSTRACT

Background: The etiology of testicular germ cell tumors (TGCT) is poorly understood, however, exposure to endocrine disrupting chemicals (EDCs) may be related to increased risk. Personal care products, some of which contain EDCs, are widely used on a daily basis and are known to cross the placenta, be present in breastmilk, and are capable of inducing reproductive tract abnormalities. To determine the association between personal care product use during pregnancy and breastfeeding and TGCT risk, an analysis among mothers of TGCT cases and controls was conducted.

Methods: The US Servicemen's Testicular Tumor Environmental and Endocrine Determinants (STEED) study enrolled TGCT cases and controls and their mothers between 2002 and 2005. The current analysis examined personal care product use during pregnancy among 527 mothers of TGCT cases and 562 mothers of controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression adjusting for identified covariates.

Results: Maternal use of face lotion more than one time per week was associated with a significantly increased risk of TGCT (OR: 1.42, 95% CI: 1.08–1.86, p-trend: 0.01). None of the other products examined (perfume, hairspray, nail polish, hair dye, permanent wave, body lotion, deodorant, sunscreen) were associated with TGCT risk.

Conclusions: Frequent exposure to face lotion during pregnancy and while breastfeeding may be associated with

Rationale & objectives

Background

Exposure to endocrine disrupting chemicals (EDC) during fetal development and puberty plays a role in the increased incidences of reproductive diseases, endocrine-related cancers, behavioural and learning problems

Objectives

- Analytical method development for EDC determination in placenta samples

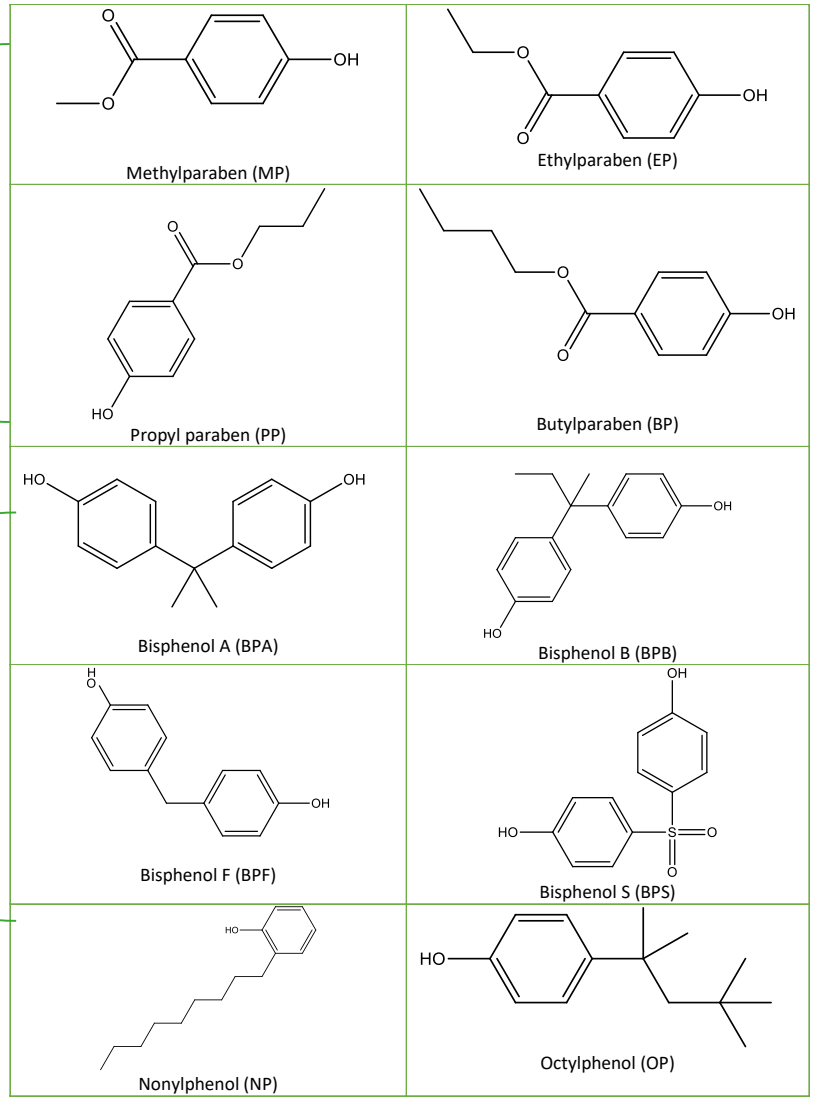
- Study the association between placental EDC levels and birth parameters in the newborns (birth weight, gestational age)
- Study the association between placental EDC levels and biochemical parameters in the newborn
- Study the association between placental EDC levels and molecular parameters in the newborn

Target compounds

4 parabens

4 bisphenols

2 alkylphenols



sciensano

.be

Methods

Samples

UPLC MS/MS

Methods-Sample origin

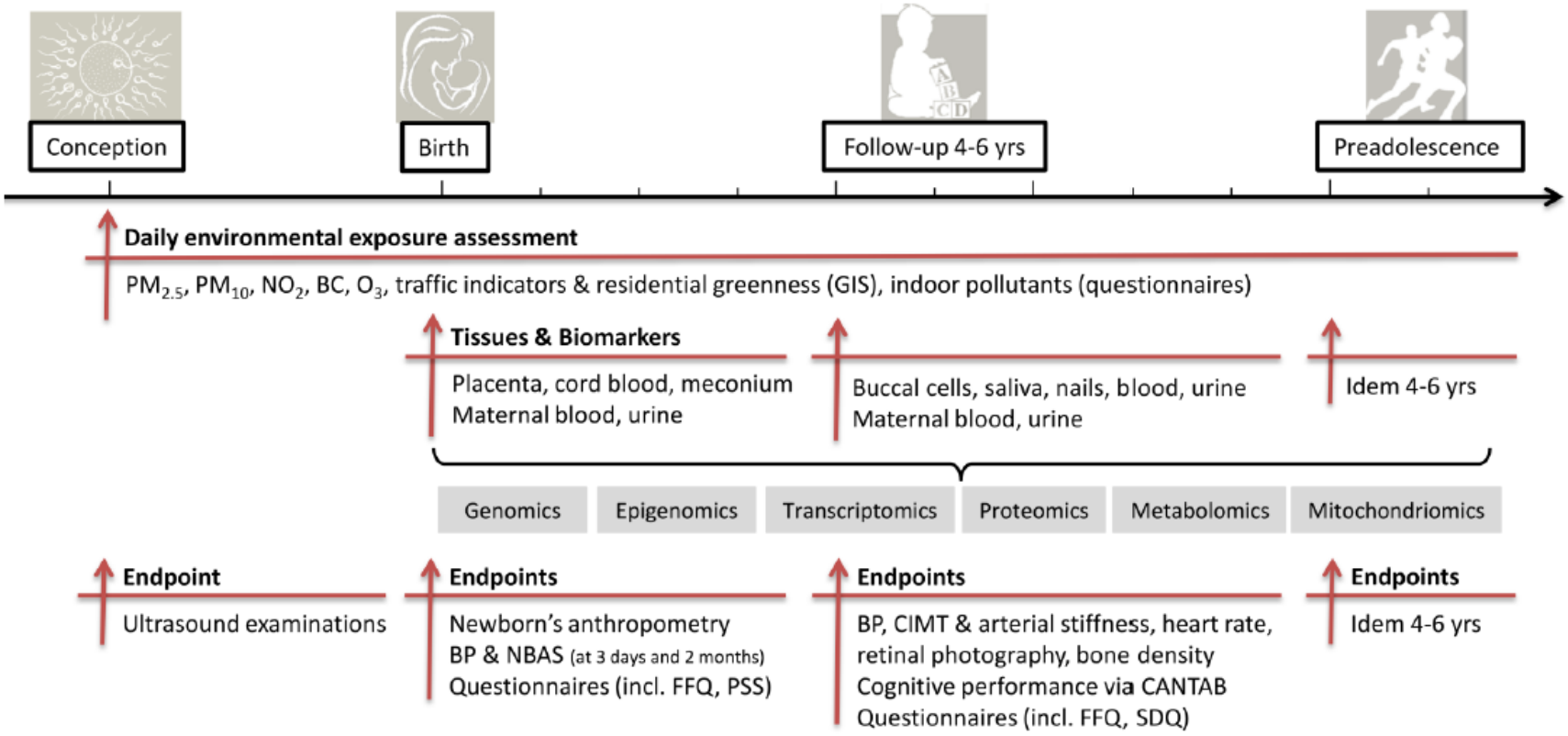
In Environage birth cohort:

1800 mother-child pairs included and recruitment is ongoing

ENVIR*on*mental Influence on AGEing: early in life exposures affect life long health of the newborn

Investigate consequences on molecular as well as clinical level

ENVIRONAGE birth cohort



sciensano

Janssen et al., 2017 .be

Method development issues

- No reference material

→spiked samples

- Avoid external contamination

→PP tubes, procedural blanks

Results-analytical method development

Sample preparation

1.5 g homogenized placenta tissue

+ isotopically labeled internal standards

Vortex mixing

Liquid-liquid extraction with tert-butyl dimethyl ether/hexane (50/50)

Centrifugation

Evaporation of supernatant

Reconstitution in H_2O/MeOH

Mass spectrometry

10 µL injection for UPLC ESI$^-$ MS/MS analysis

Column: Acquity BEH C18 (2.1 x 100mm, i.d. 1.7µm)
MS: Xevo-TQ triple quadrupole: ESI-
Gradient elution at 0.4 ml/min using 0.1% NH_4OH in water and in acetonitrile

Ref: Van Overmeire et al., J. Chrom B (2019)

sciensano

.be

Results :method validation parameters

- ✓ **Linearity** :linear range from 0.5-50 ng g^{-1}
- ✓ **Selectivity**
- ✓ **Precision**

At 3 QC levels (0.5-5 and 25 ng g^{-1})

Rsd below 20%

- ✓ **Recovery** values within 15 %

of spiked concentration)

- ✓ **Matrix effect** (matrix matched

Calibration, labeled IS)

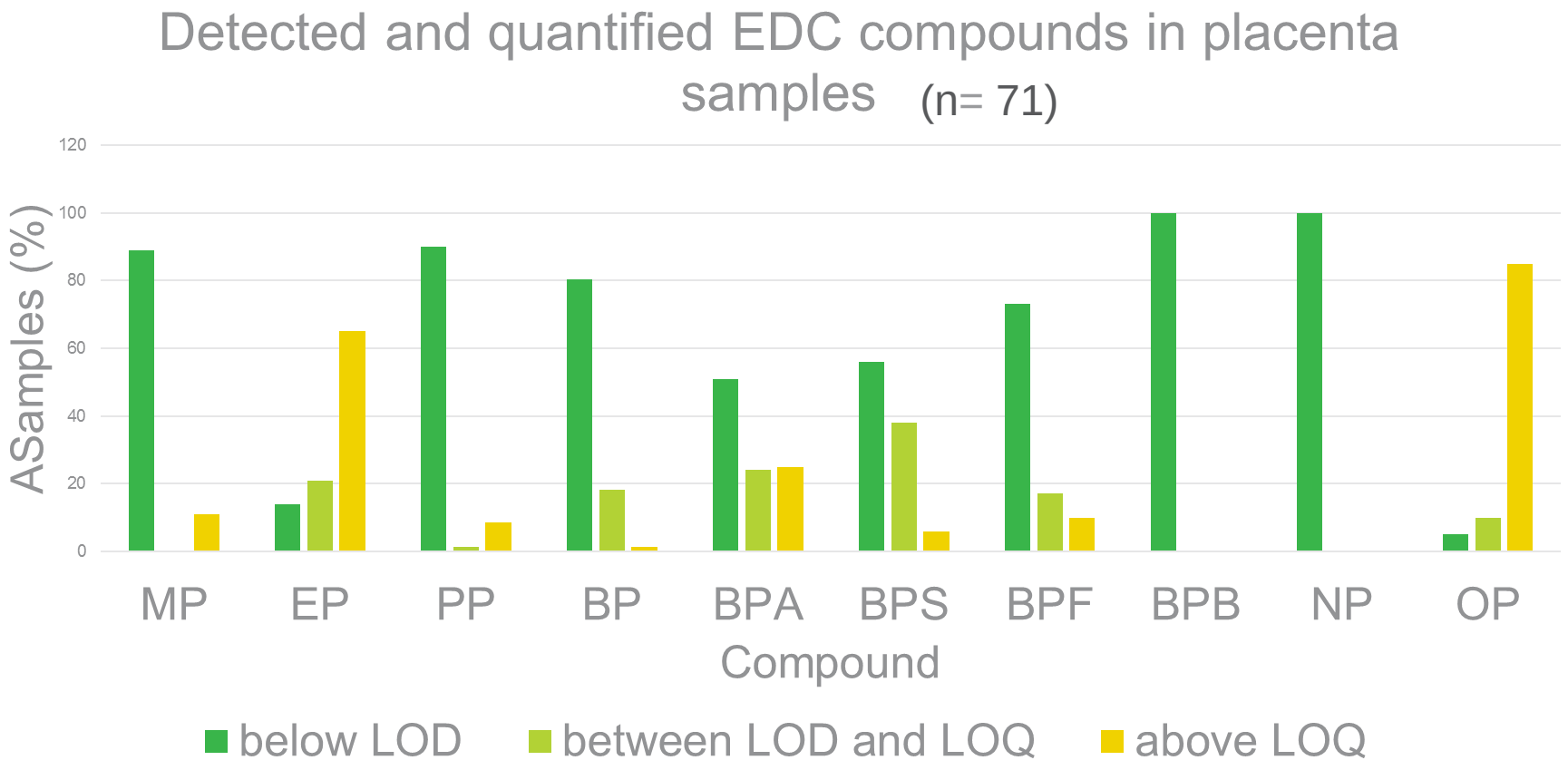
- ✓ **Sensitivity**

Compound	LOD (ng g^{-1})	LOQ (ng g^{-1})
MP	0.1	0.2
EP	0.2	0.5
PP	0.1	0.4
BP	0.2	0.4
BPA	0.2	0.4
BPS	0.3	0.7
BPF	0.2	0.6
BPB	0.1	0.4
NP	0.1	0.5
OP	0.1	0.5

Results -concentrations in human placenta samples (n = 71)

Compound	Mean (ng g^{-1})	Range (ng g^{-1})
MP	4.3	0.5-7.1
EP	1.0	0.5-4.5
PP	2.2	0.5-9.1
BP	<LOD	
BPA	0.7	0.5-3.9
BPS	0.3	0.8-1.3
BPF	0.6	0.6-2.1
BPB	-	
NP	-	
OP	1.3	0.5-3.7

Results-Detection (and quantification) rate



sciensano

.be

Comparison with other studies-parabens

compound	Mean ng g-1)	Median (ng g-1)	Det. freq/Quant .freq *(%)	Country (sample number)	LOQ (ng g-1)	Range (ng g-1)	Reference
MeP	2.6	1.6	96/94	Spain (50)	0.1	0.2-10	[Jiménez-Diaz, 2011]
	7.6	5.9	90/90	Spain (10)	0.3	0.8-16.1	[Vela-Soria, 2014]
	8.2	6.5	100/90	Spain (10)	0..3	1.0-16.8	[Vela-Soria, 2015]
			87/33	Spain (15)	0.3	0.5-1.5	[Vela-Soria, 2017]
			100/42	Spain (12)	0.06	1.2-11.8	[Valle-Sistac, 2016]
	4.3	4.4	11/11	Belgium (71)	0.2	0.5-7.1	This study
EtP	0.8	0.4	66/40	Spain (50)	0.2	0.2-5.3	[Jiménez-Diaz, 2011]
			10/10	Spain (10)	0.3	0.7	[Vela-Soria, 2014]
			20/10	Spain(10)	0.3	1.6	[Vela-Soria, 2015]
			67/40	Spain (15)	0.4	0.5-2.2	Vela-Soria, 2017]
			75/58	Spain (12)	0.02	0.1-0.6	[Valle-Sistac, 2016]
	1.0	0.7	86/65	Belgium (71)	0.5	0.5-4.5	This study
PrP	0.6	0.5	90/90	Spain (50)	0.2	0.2-2.2	[Jiménez-Diaz, 2011]
	1.8	1.9	60/40	Spain (10)	0.3	0.5-2.9	[Vela-Soria, 2014]
	1.6	0.9	60/60	Spain (10)	0.3	0.4-3.4	[Vela-Soria, 2015]
			100/0	Spain (15)	0.3		Vela-Soria, 2017]
			92/42	Spain (12)	0.01	0.1-1.3	[Valle-Sistac, 2016]
	2.2	1.0	10/8	Belgium (71)	0.4	0.5-9.1	This study
BuP	0.4	0.5	16/8	Spain (50)	0.2	0.2-0.6	[Jiménez-Diaz, 2011]
			10/0	Spain (10)	0.4		[Vela-Soria, 2014]
			10/0	Spain (10)	0.3		[Vela-Soria, 2015]
			0/0	Spain (15)	0.4		Vela-Soria, 2017]
			100/75	Spain (12)	0.02	0.0-0.9	[Valle-Sistac, 2016]
			20/1	Belgium (71)	0.4		This study

Comparison with other studies-bisphenol A

compound	Mean ng g-1)	Median (ng g-1)	Det. freq/Quant .freq *(%)	Country (sample number)	LOQ (ng g-1)	Range (ng g-1)	Reference
BPA	-	-	35/35	Spain (49)	0.5	1.1-22.2	[Jiménez-Diaz, 2010]
	9.1	8.7	50/30	Spain (10)	0.3	4.2-14.5	[Vela-Soria, 2015]
	4.1	3.5	86/86	Canada (21)	O.8	1.0-7.8	[Zhang, 2011]
	0.6	0.5	82	Korea (257)		Below LOD-53	[lee, 2018]
	0.7	0.4	49/25	Belgium (71)	0.4	0.5-3.9	This study

Next steps

- Study the determinants important in the establishment of EDC levels in placenta (parental smoking, education, BMI, season of delivery, parity..)
- Study the association between placental EDC levels and birth parameters in the newborns (birthweight, gestational age)
- Study the association between placental EDC levels and biochemical parameters in the newborn (measurements of following agents in cord blood:IL-6, homocysteine, vitamin D, estrogen, thyroid hormones (TSH, FT3, FT4)
- Study the association between placental EDC levels and molecular parameters in the newborn

Conclusions and perspectives

- Sensitive analytical method was developed
- Endocrine disrupting chemicals (EDC) could be quantified in human placenta samples
- The major compounds found were parabens and bisphenols
- Highest concentration levels for methyl- and propylparaben and highest quantification frequency for octylphenol and ethylparaben
- Bisphenol A and bisphenol S both detected and quantified
- Enlarging the number of samples will enable to correlate EDC levels in placenta with health outcomes of the born children

Acknowledgement

Sciensano own funding

University Hasselt

Dr A. Van Nieuwenhuyse

sciensano

.be